FACTORS ASSOCIATED WITH THE SYNTHESIS OF CHLOROPHYLL IN THE DARK IN SEEDLINGS OF PINUS JEFFREYI

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 611

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Introduction

All the theories proposed for the biosynthesis of chlorophyll in angiosperms include two sets of reactions: one photochemical; the other capable of proceeding in the dark. In 1859 SACHS pointed out, however, that the lower plants, including the seedlings of conifers, are able to green entirely without light. SACH's observations were extended, in 1900, by BURGERSTEIN (2), who examined about sixty species of gymnosperms and found that most of them were capable of greening in the dark. Subsequently, SCHMIDT (16) observed that the pigment formed in the dark is spectroscopically identical with that formed in the light. SEYBOLD and EGLE (17, 18) studied the course of synthesis of the chlorophylls in the germinating seeds of *Picea excelsa*, paying particular attention to the ratio of chlorophyll *a* to *b*. LUBIMENKO (11) investigated the rate of synthesis of chlorophyll in seedlings of *Pinus sylvestris* and *Picea excelsa* in darkness and in light and reported that chlorophyll was formed much more rapidly in light than in darkness. He postulated that the photochemical reaction in chlorophyll formation is the conversion of "chlorophyllogen" into chlorophyll and that plants capable of synthesizing chlorophyll in the dark carry out this step enzymatically. Most recently MEYERS (14) found the chlorophyll produced by *Chlorella* and *Protococcus* in darkness and in light to be identical spectroscopically and equally efficient in photosynthesis.

SCHMIDT (16) made certain other observations on the development of chlorophyll in germinating pine seeds. He found that embryos isolated from the megagametophyte failed to form chlorophyll when cultured on any of a number of organic or inorganic substances. He observed, too, that the intensity of green pigmentation of the embryo was a function of the length of time the entire seed had been soaked in water; the more prolonged the soaking, the greener the embryo. Replantation and transplantation experiments showed that the natural relative position of embryo and megagametophyte was not required for chlorophyll synthesis in the dark and that the megagametophyte of one species of *Pinus* was effective in promoting chlorophyll formation in the embryo of another species.

Isolation of the factors for chlorophyll synthesis in two separate tissues appeared to offer a favorable opportunity for the study of this process, particularly for the eventual elucidation of the photochemical steps which occur in those plants which green only in light. The work reported in this paper is concerned with a study of certain relationships between embryo and megagametophyte and attempts to determine the nature of the substance (or substances) acquired by the embryo from the megagameto-

phyte which facilitates the production of chlorophyll without light.

Material and methods

Seeds of *Pinus jeffreyi* were chosen as the experimental material partly because they had been used by SCHMIDT (16), but mainly because they were the largest pine seeds available, in the amounts required—about 8 pounds—at the time this work was started. A small quantity was obtained from the United States Forest Service Station at Susanville, California, but the bulk was obtained from Herbst Brothers Seed Company of New York City. Seeds of the 1947 harvest were used in most of the experiments, but seeds from the 1948 crop were used for the last experiment.

It was necessary to hasten the time for germination, to make it as uniform as possible for a large number of seeds, and to reduce bacterial and fungal contamination to a minimum. Since many of the seeds were infected and contained spores of potential contaminants, seed coats were removed, and only sound-appearing seeds were selected. The term "seed" is used hereafter in this paper as it has just been used—as a convenient term for the megagametophyte and embryo sporophyte portion in their natural relationship but with seed coats removed. The seed coat of each seed was removed by hand, since various mechanical methods failed to give a high enough proportion of undamaged seeds.

The selected seeds were sterilized in a 1% solution of bromine water for 5 minutes and were then transferred to sterile Petri dishes containing two sheets of filter paper. They were left in these dishes for from 45 to 60 minutes to permit the bromine to evaporate and the water to drain off and were then again transferred to other dishes containing the medium to be used in the experiment. Usually ten seeds were placed in each dish.

The final transfer of seeds was performed under full light, but in all experiments concerned with the synthesis of chlorophyll in the dark subsequent manipulation of seeds and seedlings was carried out under very dim green light. As soon as the desired number of seeds had been put into a dish, it was placed in an individual lighttight container made of black construction paper and black masking tape. Each container was a slightly oversized replica of a Petri dish, so designed that the space between the vertical walls of the upper and lower halves was small and so that the lower half was deeper than the height of the Petri dish. This container excluded all light to which panchromatic film is sensitive. The use of individual containers minimized the spread of contamination from one dish to another and permitted free manipulation of the dishes.

The effect of variability in time of germination was reduced by permitting the seeds to germinate under the conditions of the particular experiment until some predetermined time; those which showed about the same amount of elongation of the radicle were then selected for further use. Each replicate consisted of from five to fifteen plants.

The terms "embryo" and "seedling" are used to refer to the sporophyte: "embryo" for sporophytes excised from the ungerminated seed and cultured outside the megagametophytes; "seedling" for sporophytes which developed in contact with megagametophytes during at least part of the period of germination. The term "intact seed" is used to designate germinated or germinating seeds in which the megagametophytes and young sporophyte have not been manipulated and are in natural contact with each

other. In some experiments the contact of seedling with megagametophyte was interrupted several days after germination and then, after a period of isolation, contact was re-established. The renewed contact was made between the excised seedling and a megagametophyte of a seed which had germinated usually 2 days later than the older seedling. The younger seedling was removed from the megagametophyte, and the older seedling was put in its place.

Fresh plant material was used for all chlorophyll analyses. The water content of the tissue was determined in preliminary experiments so that the amount of acetone required for a concentration of 80% could be calculated. The procedure, for chlorophyll analysis, was to grind the tissue in a mortar under 80% acetone with a small amount of acid-washed quartz sand. The triturate was washed into a Gooch crucible fitted with filter pad. The macerate was washed with additional solvent, and the filtrate was collected in a volumetric flask and made to volume. The chlorophyll content of this solution was determined by reading its density at 635 mμ and at 660 mμ in a Beckman spectrophotometer. Concentrations of chlorophylls *a* and *b* were then calculated according to the method of COMAR and ZSCHEILE (3). The specific absorption coefficients reported by ZSCHEILE *et al.* (23) were used. Some additional details of calculations have been published recently by ARNON (1).

Magnesium determinations were made by the thiazole yellow method of DROSDOFF and NEARPASS (4). This method was modified by using a hydrochloric acid digest of dried material and by determining the density of the solutions with the Beckman spectrophotometer at 548 mμ. The details of preparation and analysis of ether extracts are given later in this paper. Total iron was determined by the *o*-phenanthroline method of SAYWELL and CUNNINGHAM (15) as modified by JACOBSON (7). The method, as used here, was further modified by reading the densities of the solutions at 510 mμ with the Beckman instrument.

The data are usually reported on an absolute basis—amount of given substance per plant—because this basis shows the change in amount of only the substance under investigation. Expression of the concentration on the basis of fresh weight—the data presented by LUBIMENKO (11) are expressed in this way—reflects changes in weight as well as in the concentration of the substance being followed. These changes may not be proceeding in the same direction or at the same rate, and results expressed on this latter basis may be misleading or difficult to interpret.

Experimentation and results

COMPARISON OF CHLOROPHYLL PRODUCTION IN PRESENCE AND ABSENCE OF LIGHT

SPECTROSCOPIC COMPARISON OF PIGMENTS.—A spectroscopic study was made of 80% aqueous acetone extracts of green pigments from sporophytes grown under different conditions of nutrition and light. The absorption spectrum for embryos excised from ungerminated seeds (curve *3*, fig. 1) shows slight maxima (really plateaus in the curve) at 420–422 mμ, 426–430 mμ, and 460–470 mμ, suggesting the presence of carotenoids. The slight rise at 663–668 mμ indicates the presence of a small amount of chlorophyll. All the embryos used were colorless to the eye, but, since about 0.1% of these seeds contain embryos which are green prior to germination, the detection of a small amount of

chlorophyll is not surprising. Curve *2* is for excised embryos grown on Loo's medium (10 and table 1) in darkness for 8 days. This spectrum shows absorption maxima at 422, 440, and 470 mμ typical of the carotenoids, and a very slight rise in the red end of the spectrum, similar to that displayed by the ungerminated embryos. The absorption spectrum for excised embryos grown on Loo's medium in the light for 8 days (curve *1*) clearly shows the presence of chlorophyll by absorption maxima at 435 mμ and especially at 663 mμ. Thus, on Loo's medium, excised embryos grown in the light developed chlorophyll, while those grown under similar conditions but without light did not.

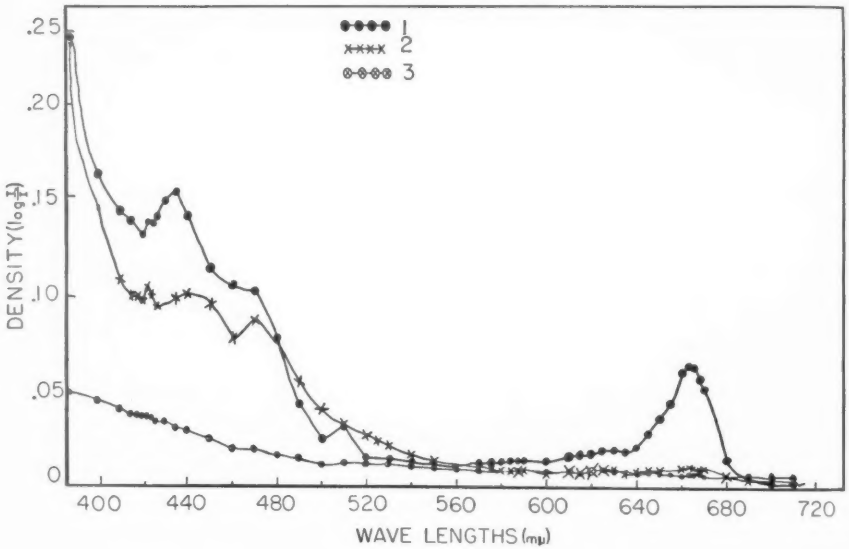


FIG. 1.—Absorption spectra of 80% aqueous acetone extracts of: *1*, excised embryos grown in light on Loo's medium (8 days old); *2*, excised embryos grown in dark on Loo's medium (8 days old); and *3*, embryos excised from ungerminated seeds.

TABLE 1

COMPOSITION OF LOO'S MEDIUM

$Ca(NO_3)_2$	328	mg/liter
KNO_3	80	mg/liter
$MgSO_4 \cdot 7H_2O$	98	mg/liter
$KHSO_4$	13	mg/liter
KCL	74	mg/liter
$FeCl_3$	1.6	mg/liter
Heteroauxin	10.0	mg/liter
Thiamine hydrochloride	0.1	mg/liter
Sucrose	20.0	gm/liter
Agar	7.0	gm/liter

Figure 2 shows the absorption spectra of 80% acetone extracts of seedlings grown in the dark from intact seeds (curve *3*); of excised embryos grown on autoclaved megagametophytes and agar in the light (curve *1*); and of excised embryos grown in the dark on a buffered aqueous extract (pH 6.8) of megagametophytic tissue (curve *2*). Curves *1* and *3* are of particular interest for comparing the pigments produced by embryos in light without living megagametophyte and by those in darkness in contact with

this tissue. Both of these curves show the presence of chlorophyll; the differences between them may be attributed to differences in the concentrations of *a* and *b* components. Excised embryos grown without light on a medium composed of autoclaved megagametophytic tissue and agar developed no chlorophyll.

Absorption spectra were similar for diethyl ether solutions of chlorophyll *a* isolated from seedlings grown from intact seeds in the dark and from seedlings

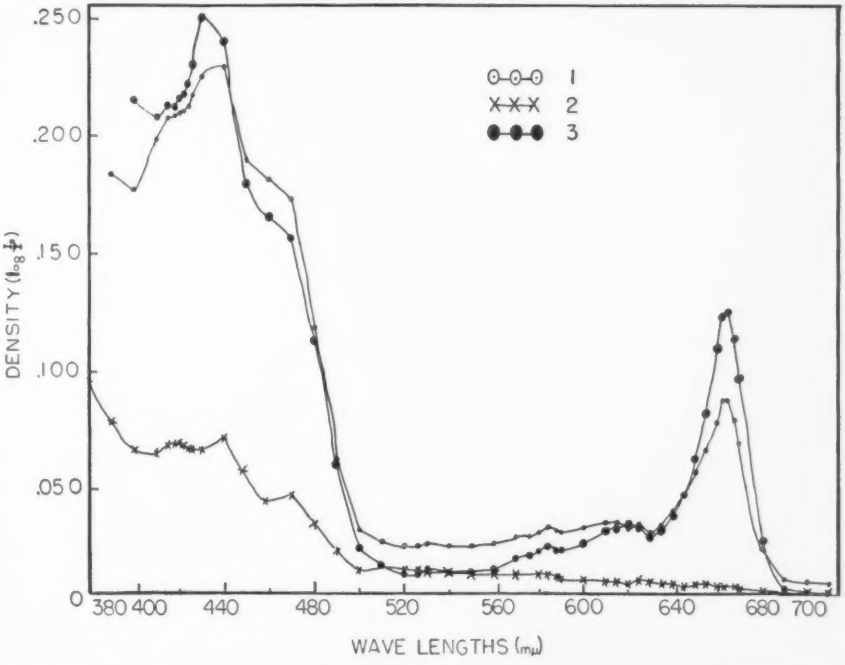


FIG. 2.—Absorption spectra of 80% aqueous acetone extracts of: *1*, excised embryos grown on autoclaved megagametophyte and agar medium in light; *2*, excised embryos grown on aqueous extract of megagametophytic tissue in dark; and *3*, seedlings grown from intact seeds in dark.

Absorption spectra were determined for solutions of chlorophylls *a* and *b* isolated from sporophytes grown in darkness in contact with megagametophytic tissue. The isolation procedures described by ZSCHEILE and COMAR (**22**) were followed. Major absorption maxima for chlorophyll *a* in diethyl ether were found at 660 and 429 mμ, and for chlorophyll *b* at 642.5 and 453 mμ. Eighty per cent aqueous acetone solutions of chlorophyll *a* obtained from similar seedlings showed an absorption spectrum which corresponded closely to that reported by MACKINNEY (**13**).

grown in the dark under these conditions for 6 days and then placed in the light without megagametophytic tissue for 6 days. Both showed major absorption maxima at 429 and 660 mμ.

These data support the observations of SCHMIDT (**16**) that the embryo sporophyte of *P. jeffreyi* produces chlorophyll in the dark when in contact with the megagametophyte but fails to do so when removed from it and that the chloro-

phylls produced in the dark and in the light are similar spectroscopically.

Excised embryos, not in contact with living megagametophytic tissue and with or without light, were inferior in over-all length, growth of cotyledons, and gain in dry weight to embryos which had developed in contact with their megagametophytes in the dark during an equal or shorter period of time. These differences can probably be explained by differences in the availability of certain required growth-regulating substances or nutrients present in, or produced by, the living megagametophyte but not supplied to excised embryos by any of the media used in these experiments. For this reason, in subsequent experiments the embryos were generally allowed to develop for a time in contact with their megagametophytes before being separated from them or being subjected to other experimental conditions.

QUANTITATIVE COMPARISON OF CHLOROPHYLL PRODUCTION IN LIGHT AND DARKNESS.—The following experiment (table 2 and fig. 3) was designed to study the relative effects of light and of megagametophyte on chlorophyll formation in the seedling and to determine whether the dark synthesis could be resumed after the seedlings had once synthesized chlorophyll in the light.

About 1000 seeds were sterilized and transferred aseptically into Petri dishes containing 0.7% washed agar. All the seeds were germinated at 23° C. in the dark for 6 days. Seeds which had germinated to about the same extent were then selected for further use. Thirty-two plants were selected at random, and the chlorophyll content of the seedlings was determined immediately. The other germinating seeds were divided into three groups: (*1*) Seeds were transferred directly to Petri dishes containing 0.7% agar and returned to total darkness. (*2*) and (*3*) Seedlings were separated from the megagametophytes and transferred to fresh 0.7% agar medium. Half of these (*2*) were returned to darkness, and the other half (*3*) were placed under continuous illumination provided by a "daylight" fluorescent tube. Light intensity at the surface of the dishes was 60 foot-candles. The plants in all three groups were maintained at the same temperature.

On the second and fourth days after the beginning of differential treatment—8 and 10 days after the beginning of the experiment—samples for chlorophyll analysis were taken from all three groups. Also on the fourth day seedlings of group *3* which had been under continuous illumination were returned to darkness, some after being placed in contact with megagametophytes from other seeds (*3a*) and the remainder without this treatment (*3b*). Those seedlings of group *2* which had been in the dark for 4 days were divided into two subgroups: one was placed in the light (*2a*), and the seedlings of the other were placed in contact with megagametophytes and kept in the dark (*2b*). The seeds of group *1* were not treated in any way. On the second and fourth days following this new treatment—the sixth and eighth days following the initiation of treatment—samples were drawn from each group for chlorophyll analyses.

Twenty-four-hour illumination daily was used in all light treatments. This treatment was employed rather than one of alternating periods of light and darkness because in the parallel treatments—the seedling in contact with the megagametophyte in darkness—the substances contributing to chlorophyll formation presumably were being trans-

ferred from the megagametophyte to the seedling all the time.

The data (table 2 and fig. 3) show that, during the first 2 days after separation from their megagametophytes, seedlings continued to produce chlorophylls *a* and *b* (fig. 3) in darkness. The rate of synthesis in excised seedlings grown in the light during this period, however, was 4.6 times greater than in those in the dark. The rate of chlorophyll synthesis in the dark in seedlings from intact seeds was slightly lower than in excised seedlings in the light, but the difference is not statistically significant. Thus, under conditions of this experiment, illumination of seedlings isolated from their megagametophytes resulted in a net increase in chlorophyll content about equal to that in seedlings of the same age which continued in contact with their megagametophytes in the dark and in a fourfold increase over the rate of chlorophyll production in excised seedlings in the dark. The last is probably the more valid comparison, because the excised seedlings, in light and darkness, were under approximately similar nutritional conditions. Figure 4 reflects this latter point in that fresh weights of excised seedlings in the light (curve 3) were very close to those for similar seedlings in the dark (curve 2), whereas the weights for seedlings in the dark in contact with their megagametophytes (curve 1) show a much greater increase than do the weights of either of the other two groups.

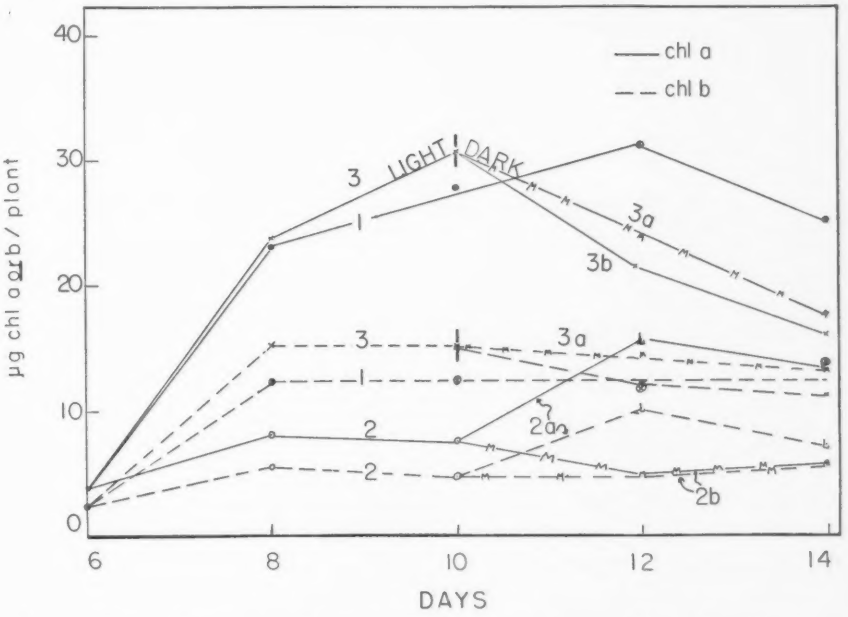


FIG. 3.—Chlorophyll synthesis in light and darkness. Light intensity: 60 foot-candles. Temperature: 23° C. Data given separately for chlorophylls *a* and *b*. *1*, seedlings grown from intact seeds in dark. *2*, excised seedlings grown in dark; *2a*, excised seedlings in light; *2b*, seedlings kept in dark but placed in contact with megagametophytes. *3*, excised seedlings grown in light; *3a*, seedlings returned to darkness and contact with megagametophytes; *3b*, returned to darkness without megagametophytes.

The return to darkness of excised seed-

lings which had been exposed to light had striking effects on the chlorophyll content of these seedlings, especially as compared with those which had not been exposed to light. During the 4 days following the return to darkness isolated seedlings lost 40% of their total chlorophyll, and seedlings in renewed contact with their megagametophytes lost 32.9% (table 2). The plants in none of the other treatments showed such loss. In a later experiment excised seedlings grown in the dark for 14 days after separation showed an increase in total chlorophyll during the first 2 days, no change on the sixth and eighth days, and a loss of 28.6% between the eighth and fourteenth days only.

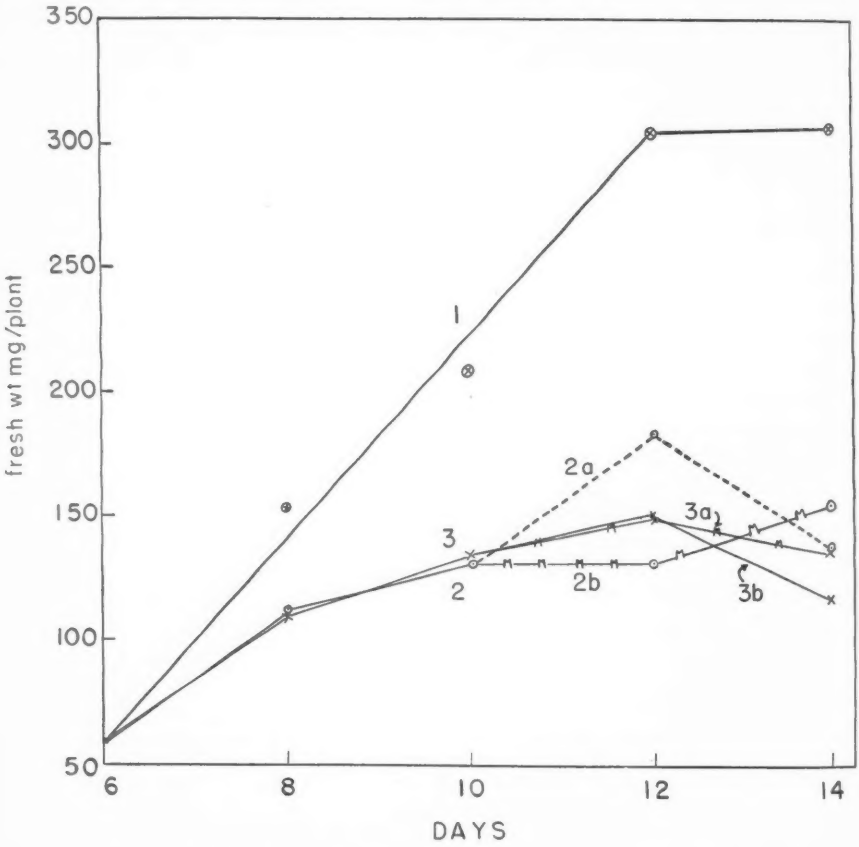


FIG. 4.—Fresh weight of seedlings grown in light and dark. See legend for fig. 3

No great increase in the rate of chlorophyll synthesis in the dark resulted when excised seedlings, which had been in the dark, were returned to contact with megagametophytes (table 2).

The lack of any really significant effect of renewed contact with the megagametophyte on chlorophyll synthesis by the

seedling in the dark may have resulted from failure to re-establish effective contact. The data provide no clear evidence that chlorophyll synthesis can or cannot occur in the dark after the excised seedlings has been exposed to light and then returned to contact with the megagametophyte. The only indication that effective contact had been re-established was provided by fresh weights of the seedlings (fig. 4). Excised seedlings kept in the dark for 4 days after separation from the megagametophytes were 16.4% heavier following 4 days of renewed contact with the megagametophytes in the dark (curve *2—M*, fig. 4). When seedlings which had been exposed to light for 4 days after separation from their megagametophytes were returned to the dark, after 4 days those in renewed contact with the megagametophytes were 14.4% heavier than those without this contact (curves *3* and *3—M*, fig. 4).

Seedlings placed in light directly after separation from the megagametophytes at 6 days synthesized chlorophyll 2.5 times more rapidly than plants subjected to a 4-day interval of darkness—after excision at 6 days—prior to exposure to light. This relationship obtained during the first 2 days of exposure to light. During this interval the rate of synthesis was 17.8 μgm. chlorophyll/seedling/day for the former treatment and 7.1 μgm. chlorophyll/seedling/day for the latter. The maximum chlorophyll content attained under the former treatment was 46.4 ± 5.8 μgm. per plant, under the latter 26.7 ± 5.5 μgm. per plant (table 2), a difference of 42.4%. This difference can be attributed to neither differences in available minerals (e.g., magnesium, iron) nor in respirable substrate, for by the nature of the experiment only those plants which remained in contact with the megagametophytes continued to receive such substances directly (cf. curve *1* with curves *2* and *3*, fig. 4), and the intensity and duration of light to which the plants were exposed were equal. The two sets of plants were, however, of different ages at the time of exposure to light.

For purposes of comparison of results obtained here with those of LUBIMENKO

TABLE 2

EFFECT OF LIGHT ON RATE OF CHLOROPHYLL SYNTHESIS
CHLOROPHYLL CONTENT IN μGM PER PLANT

Days*	Seedlings from intact seeds in dark (*1*)	Seedlings grown in contact with megagametophyte in dark and separated on sixth day			
		Light (*3*)		Dark (*2*)	
6	6.2±1.7	6.2±1.7		6.2±1.7	
8	35.1±6.4	41.7±1.3		13.9±1.6	
10	41.1±2.7	46.4±5.8		12.5±1.1	
		Dark−M†(*3b*)	Dark+M†(*3a*)	Light−M†(*2a*)	Dark+M†(*2b*)
12	44.2±3.9	33.7±2.8	38.7±4.8	26.7±5.5	9.7±0.36
14	38.5±5.4	26.9±8.1	31.1±3.8	18.6±1.6	11.2±0.98

*"Days" from beginning of experiment. Differential treatments were begun at 6 days.
† Megagametophyte.

(11), some data were calculated on the basis of μgm. of chlorophyll per mg. fresh weight. Objections to this basis of calculation have been stated earlier. At the initiation of treatment (sixth day of the experiment), the seedlings contained 0.094 μgm. of chlorophyll per mg. fresh weight. Excised seedlings exposed to light immediately after separation from their megagametophytes contained 0.380 μgm. of chlorophyll per mg. fresh weight after 2 days in the light. Seedlings grown from intact seeds in the dark contained 0.228 μgm. per mg. fresh weight at the end of the same period. Thus, the rate of chlorophyll synthesis of the former was 2.13 times the latter on this basis.

Megagametophyte-sporophyte relationships

The purpose of the following experiment was to examine the natural relationships between megagametophyte and sporophyte with regard to exchange of magnesium and iron and to study changes in dry weight accompanying germination and chlorophyll synthesis in the dark.

Approximately 1000 seeds were used. After removal of seed coats the seeds were sterilized, transferred to Petri dishes containing a medium of 0.7% washed agar in Pyrex-distilled water, and placed in darkness for 4 days. Germinating seeds of uniform size were then selected and transferred to dishes containing fresh 0.7% agar medium. On the fourth, sixth, eighth, tenth, and twelfth days of the experiment, groups of seeds were removed for chlorophyll analysis of the seedlings. On the fourth, seventh, ninth, and twelfth days, seeds were removed for determination of dry weight of seedlings and megagametophytes. The dried plants were used for total magnesium and iron analysis. In all cases determinations were made on cotyledons and hypocotyls separately.

Changes in dry weight of seedling and megagametophyte reflect the total transfer of material, minus losses due to respiration. The seedlings (fig. 5) showed an increase in dry weight about equal to the loss in dry weight exhibited by the megagametophytes during germination. The course of dry-weight increase of the cotyledons was about the same as that of the hypocotyl, except for the period of the ninth to twelfth day, when the latter showed greater increase. The weight of the hypocotyl exceeded that of the cotyledons at all points (curves *H* and *C*, fig. 5). Since, after the sixth day, the seedling was in contact with the megagametophyte only through the cotyledons, all materials acquired by the hypocotyl came via the cotyledons.

The increase in chlorophyll content of entire seedlings grown from intact seeds in the dark (fig. 6) follows that for dry weight during the same interval (fig. 5), but there seems to be no correlation between dry weight and chlorophyll increase in the hypocotyl.

The increase of magnesium in the seedlings grown from seeds in the dark is about equal to the loss by the megagametophytes (fig. 7). Separate data for the cotyledons and hypocotyl, given for the ninth and twelfth days, indicate that during this period most of the magnesium is accumulated in the cotyledons.

Also, as in the case of changes in dry weight, the curve for magnesium content of the seedlings is in general agreement with the curve for chlorophyll content, especially in the interval from the fourth to the ninth day. The increase in magnesium of the cotyledons (curve *C*, fig. 7) from the ninth to the twelfth day is not in agreement with the curve for chlorophyll synthesis, indicating perhaps some

other limiting factor in this interval. The general agreement between magnesium content of the seedling and its chlorophyll content was confirmed in a later experiment. The data also indicate that the rate of chlorophyll synthesis in the dark was slower than the rate of transfer of magnesium.

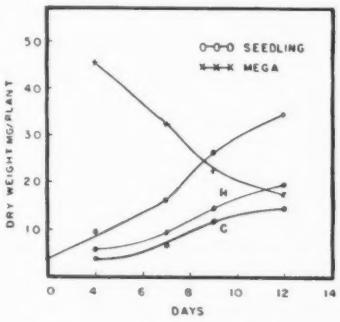
The amount of magnesium present in embryos from ungerminated seed is almost fifteen times that calculated to be present in all the chlorophyll, even in the oldest (12-day) seedlings examined (curves *S* and *a*, fig. 7).

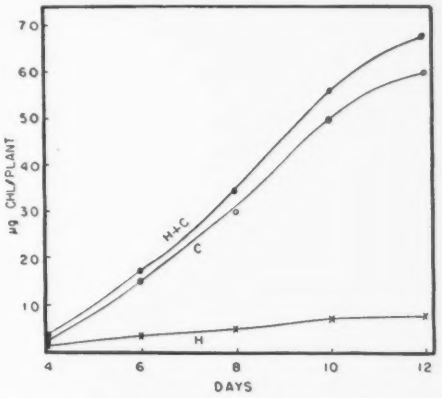
The relation of total iron content of the growing seedling and megagametophyte in contact with each other in the dark (fig. 8) repeats the relationships between these seed parts for dry weight and magnesium.

Feeding experiment

In the experiment on the quantitative effect of light and of the megagametophyte on chlorophyll synthesis in light and darkness, it was observed that seedlings separated from their megagametophytes and grown in the dark on a "non-nutrient medium" continued to produce chlorophyll for a few days. Feeding experiments were therefore performed with such seedlings to determine whether the limiting factor in chlorophyll synthesis in the dark in isolated seedlings might be respirable substrate or mineral constituents of the chlorophyll molecule.

The 2200 seeds used in this experiment were sterilized and placed in Petri dishes containing 0.7% washed agar in Pyrex-distilled water and kept in darkness for 4 days. The cultures were then examined, and germinating seeds were selected for further use. Germinating seeds were withdrawn on the fourth, sixth, tenth, and twelfth days. The seedlings were used for chlorophyll, dry weight, and magnesium determinations. On the sixth day some seedlings were separated from megagametophytes, and two treatment series were established using these seedlings: one on the washed agar medium, and the other on Loo's medium (table 1). Samples of these series were taken on the eighth, twelfth, fourteenth, and twentieth days of the experiment for chlorophyll analysis and on the ninth, twelfth,





FIGS. 5–6.—Seedlings and megagametophytes from intact seeds germinated in dark. *H*, hypocotyl; *C*, cotyledons. Fig. 5 (*left*), changes in dry weight of megagametophytes and seedlings during germination. Fig. 6 (*right*), chlorophyll content of seedlings.

and twenty-fifth days for determination of dry weights and mineral content.

Excised seedlings grown in the dark on Loo's medium showed a statistically significant increase in dry weight and magnesium content over seedlings analyzed at the time of separation (sixth day). Excised seedlings grown on the "non-nutrient medium" showed no significant changes (table 3). If the maximum amount of chlorophyll formed or its rate of synthesis by excised seedlings in the dark is limited by availability of (*a*) an energy source, (*b*) inorganic magnesium, or (*c*) the other elements present in the medium, the course of chlorophyll syn-

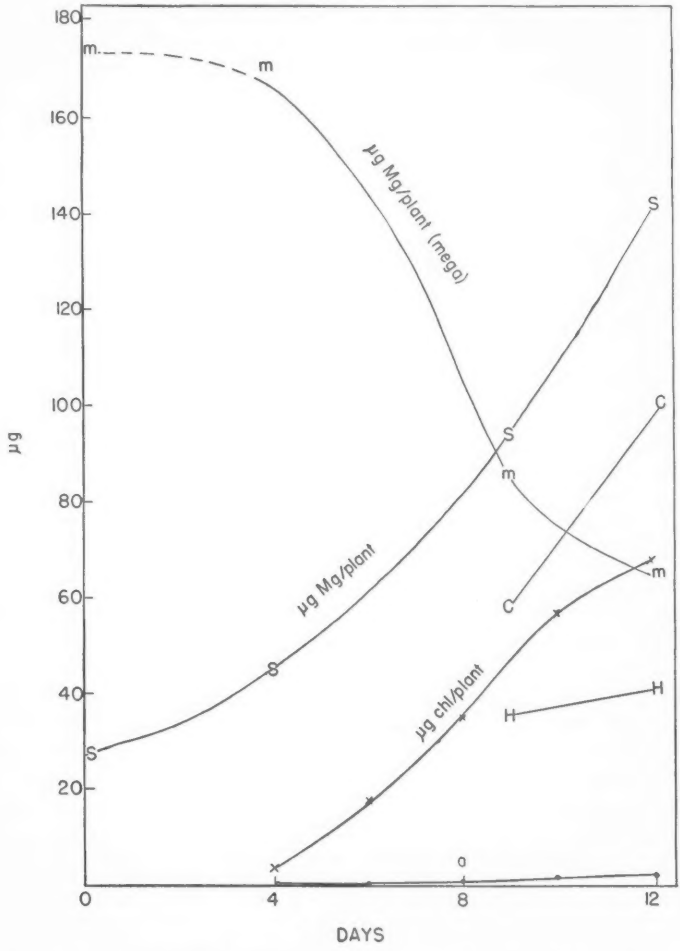


FIG. 7.—Magnesium relationships between megagametophyte and seedling during germination of seed in dark and relationship between magnesium uptake and chlorophyll synthesis by seedling growing in contact with megagametophyte in dark. Total magnesium in μgm. per plant; *m*, megagametophyte; *S*, seedling; *C*, cotyledons; *H*, hypocotyl. Magnesium present in chlorophyll in seedling calculated and shown by *a*.

thesis in the two treatments should differ. Figure 9 shows, however, that it is about the same in seedlings of the two groups.

Ether-soluble magnesium and chlorophyll synthesis in dark

Data presented by Smith (**19, 20**) suggest some relationship between changes in ether-soluble magnesium and chlorophyll synthesis in etiolated barley seedlings exposed to light. The preceding experiment was arranged to permit the use of the solutions prepared for chlorophyll determinations for the analysis of total ether-soluble magnesium.

Four replicates of approximately fifteen seedlings each were used for each determination. After the chlorophyll had been extracted with 80% acetone, the volumetric flask used as a receiver was replaced with a larger flask, and the pulp was further extracted with 50 ml. of diethyl ether, 50 ml. 80% acetone, 50 ml. Skellysolve B, and 50 ml. 80% acetone.

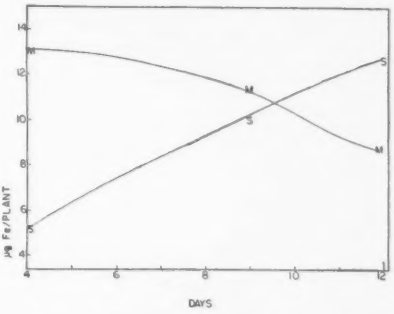


Fig. 8.—Total-iron relationship between seedling and megagametophyte during germination of intact seed in dark. *S*, seedling; *M*, megagametophyte.

This second fraction was set aside. After the chlorophyll content of the first extract—containing all the pigment—had been determined with the Beckman in-

TABLE 3

Data for seedlings grown in dark

Days*	Seedlings from intact seeds	Seedlings excised on sixth day grown on:	
		Loo's medium	Non-nutrient medium
	Dry weight†		
6	12.63±0.78	12.63±0.78	12.63±0.78
9		13.19±0.89	14.40±1.15
10	33.64±1.75		
12		13.65±1.93	9.30±1.00
25		18.30±0.70	12.27±0.66
	Total magnesium content‡		
4	47.07±2.14		
6	65.36±6.24	65.39±6.24	65.39±6.24
10	122.62±4.49		
12	127.39±7.68	84.56±2.96	53.57±2.41

* Days from beginning of experiment.
† Dry weight in mg./plant (± SE).
‡ Magnesium in μgm./plant (± SE).

strument, the two extracts were combined. The combined extract was washed with two 200-ml. portions of distilled water and two 200-ml. portions of saturated sodium sulfate and finally dried over anhydrous sodium sulfate. The dry solution was decanted from the sulfate, concentrated to small volume on a steam bath, and transferred quantitatively to a 50-ml. Erlenmeyer flask. All the solvent was then boiled off on the steam bath, and the flask was placed in a steam drying-oven for about 2 hours. One milliliter of concentrated sulfuric acid was then added, and the residue was digested (9). The digest was transferred to a 50-ml. volumetric flask and made to volume. Aliquots of this solution were used for determination of ether-soluble magnesium by a modification of the method of DROSSDOFF and NEARPASS (4).

Extraction of ether-soluble magnesium from megagametophytes was carried out as above, except that no separate extracts were made for pigment analysis, 2 ml. of sulfuric acid was used in the digestion, and the final solution was made to a volume of 100 ml.

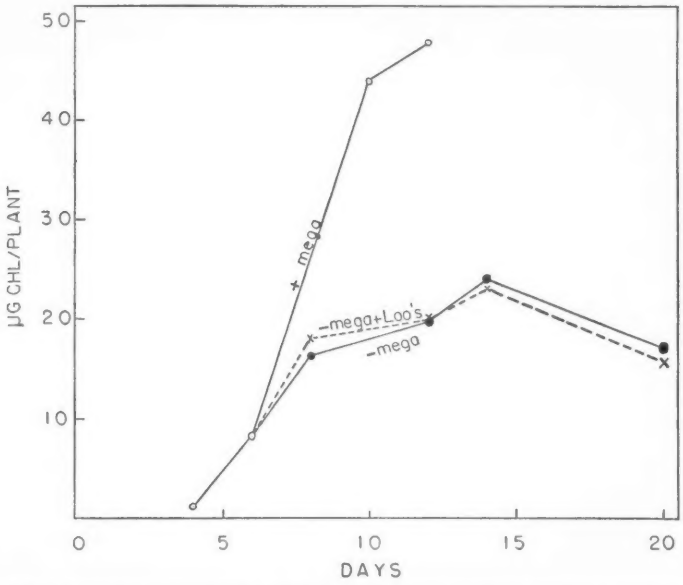


FIG. 9.—Effect of available substrate on synthesis of chlorophyll in excised seedlings grown in dark. Three treatment series begun on sixth day. Prior to this time all seedlings grown in contact with megagametophytes in dark.

The extraction with acetone and ethers alternately follows closely the procedure described by SMITH (19). Other manipulations differ.

Ether-soluble magnesium increased with time in seedlings in contact with megagametophytes in the dark, but chlorophyll increased at an even greater rate. On the fourth day of the experiment the magnesium calculated to be present in chlorophyll accounted for 2.5% of the total ether-soluble magnesium, while on the tenth day it accounted for 35.2% (table 4).

Two days after separation from their megagametophytes, excised seedlings, grown on Loo's medium in the dark, showed an increase of 0.27 μgm. of ether-soluble magnesium per plant. On the sixth day after separation there was less than at the time of separation—2.04 μgm. of ether-soluble magnesium per plant. On the second and fourth days after separation the percentage of ether-soluble magnesium attributable to chlorophyll was 19.4% and 22.0%, respectively, both well below the 35.2% maximum attained by "attached" seedlings. from the seedlings of the second group, and the seedlings were placed on fresh media and permitted to grow for 2 days in the dark before being analyzed.

The results of this experiment are shown for total chlorophyll and for chlorophylls *a* and *b* separately in figure 10. The greatest rate of chlorophyll synthesis in seedlings from intact seeds in the dark occurred in the interval between the eighth and tenth days, while the most rapid chlorophyll formation in separated seedlings was in those separated on the eighth day. Seedlings separated at times

TABLE 4

TOTAL ETHER-SOLUBLE MAGNESIUM IN SEEDLINGS GROWN IN CONTACT WITH MEGAGAMETOPHYTES

AGE IN DAYS	μGM. ETHER-SOL. Mg/PLANT ± SE	CHL. μGM./PLANT		μGM. Mg IN CHL.	% TOTAL ETHER-SOL. Mg IN CHL.
		a	*b*		
4	1.46 ± 0.26	0.8	0.5	0.04	2.5
6	2.24 ± 0.10	5.9	2.5	0.23	10.2
10	3.38 ± 0.43	31.3	12.6	1.19	35.2
12	3.74 ± 0.43	33.4	14.3	1.29	34.6

FACTORS INFLUENCING RATE AND AMOUNT OF CHLOROPHYLL SYNTHESIS IN EXCISED SEEDLINGS IN DARK

The following experiments were conducted in an attempt to learn something about the nature of the reactions leading to chlorophyll synthesis in the dark by determining if, and in what manner, temperature and time of separation of the seedling from the megagametophyte influence the rate and extent of these reactions in excised seedlings.

TIME OF SEPARATION.—Intact seeds were germinated on a 0.7% washed agar medium for 12 days. On the fourth, sixth, eighth, and tenth days two groups of seeds were removed. One group was used immediately for chlorophyll analysis. The megagametophytes were separated between the fourth and the eighth days appeared to synthesize chlorophyll more rapidly the longer they had previously been in contact with the megagametophytes under conditions conducive to germination. Seedlings separated on the tenth day did not follow this trend. This may be correlated, perhaps, with exhaustion of certain constituents of the megagametophyte and the reduction of the differences between the rates of transfer of material and incorporation into the chlorophyll molecule.

An experiment was conducted to determine the effect of time of separation on the maximum net gain in chlorophyll. The procedure followed was essentially that of the preceding experiment except that larger numbers of seedlings were

separated at each point, and analyses were made at several times after separation. Seedlings separated on the eighth day after the beginning of the experiment gained 1.6 times more chlorophyll than those separated on the sixth day (table 5).

TEMPERATURE COEFFICIENT.—The influence of temperature on the rate of chlorophyll formation by excised seedlings in the dark was determined.

About 500 seeds were sterilized, put into Petri dishes containing a medium of 0.7% washed agar in distilled water, and placed in the dark. On the sixth day the seedlings were separated from their megagametophytes and transferred to Petri dishes with fresh media. Twenty-four seedlings were used immediately for chlorophyll analysis, and the remainder was divided into three groups: the first was placed in a chamber thermostatically controlled at 11° C., the second at 23.5° C., and the third at 37° C. Forty-eight hours later the three groups, each consisting of five replicates with eight to ten plants per replicate, were analyzed for chlorophyll content.

Previous evidence indicated that the reaction rate is linear over this time interval, and yet the period is long enough to reduce the effect of time of separation. (The separation, under sterile conditions, of the number of seedlings required in an experiment of this size takes two people about 3 hours.)

Temperature coefficients were calculated for the intervals 11°–23.5° C. and 23.5°–37° C. by the following formula:

$$Q_{10} = \left(\frac{\text{Rate at } t_2}{\text{Rate at } t_1}\right)^{10/n},$$

where n = the actual temperature interval in degrees centigrade. The coefficient for total chlorophyll production in the dark in the 11°–23.5° C. interval was

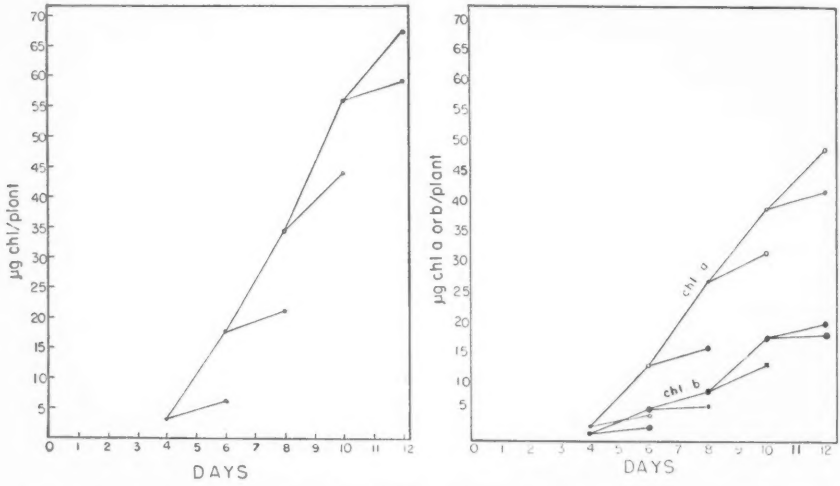


FIG. 10.—Effect of time of separation of seedling from megagametophyte on rate of chlorophyll synthesis by excised seedling in dark. Continuous curves, from 4th to 12th days, show course of chlorophyll production in seedlings from intact seeds in dark. Lines shown leaving main curves on 4th, 6th, 8th, and 10th days represent course of chlorophyll increase in seedling during 2 days following separation. *Left*, total chlorophyll. *Right*, chlorophylls *a* and *b* shown separately.

found to be 1.69. Separate calculations for chlorophylls *a* and *b* gave values of 2.37 and 1.31, respectively, in this interval. The coefficient for total chlorophyll production in the dark in the interval 23.5°–37° C. was found to be 0.128, suggesting, perhaps, that the rate of chlorophyll destruction approaches that of synthesis in this range. Another experiment was performed over part of this range, at 23.5° and 34° C. The time from separation to analysis was reduced from 48 to 36 hours. The temperature coefficient under these conditions and in this interval was 0.326.

TABLE 5

EFFECT OF DURATION OF CONTACT BETWEEN SEEDLING AND MEGAGAMETOPHYTE ON MAXIMUM NET GAIN IN CHLOROPHYLL IN DARK

SEEDLINGS FROM INTACT SEEDS		EXCISED SEEDLINGS†			
Days*	μgm. chl./seedling	Excised on	Days after excision	μgm. chl./seedling	Max. net gain μgm. chl./seedling
4	5.36				
6	16.84	6th day*	11	22.32	
8	31.17		14	22.33	5.49
10	37.36	8th day*	6	36.45	
14	51.22		9	40.02	8.85
			12	39.08	

*"Days" from beginning of experiment.
† Grown on 0.7% washed agar in Pyrex-distilled water.

Discussion

Seedlings of *P. jeffreyi* grown in the dark in contact with their megagametophytes developed chlorophyll pigments spectroscopically similar to those formed by embryos excised from ungerminated seeds and grown on a synthetic medium (table 1) in the light. Excised embryos grown in the dark on the same synthetic medium, on aqueous extracts of megagametophytic tissue, or on a medium of autoclaved megagametophytes and agar, did not form chlorophyll. Also, SMITH and KOSKI (**21**) reported recently that cotyledons of *P. jeffreyi* which emerged in the dark contained chlorophylls *a* and *b* but that the new growth of leaves and stems produced by 20-inch tall pine saplings in the dark was nearly colorless. These new needles were found to contain traces of chlorophylls *a* and *b* and also protochlorophyll. Thus, in this species, at least one step in chlorophyll synthesis is a photochemical one for which a non-photochemical reaction(s) may be substituted by the presence of the megagametophyte.

Considerable amounts of materials from the megagametophyte are transferred to the seedling during germination of intact seeds (figs. 5, 7, 8). The observation (*a*) that the maximum net gain in chlorophyll made by seedlings separated from their megagametophytes and grown in the dark depends on the preceding duration of contact during germination (table 5) and (*b*) the general agreement in the shapes of the curves for chlorophyll content and dry-weight changes (figs. 5, 6) seem to indicate that the transfer of some material from the megagametophyte is involved in the lack of

necessity for photochemical steps. The curves for chlorophyll synthesis and total magnesium transfer show good agreement. The amount of magnesium present in the embryos excised from ungerminated seeds, however, is far in excess of the total ether-soluble magnesium, including that in chlorophyll, found in the seedlings of any age studied during the experiment (fig. 7). Furthermore, feeding experiments with excised, partially greened seedlings showed that the availability of inorganic magnesium was not a limiting factor in chlorophyll synthesis in the dark (fig. 9). It may well be that the incorporation of inorganic magnesium into an ether-soluble organic compound can be performed by the megagametophyte and not by the seedling in the dark. SMITH (**19, 20**) has shown that this is a photochemical process in etiolated barley seedlings exposed to light and is probably related to chlorophyll synthesis. There is, however, no direct evidence for this action by the megagametophyte. Data on the amount of ether-soluble magnesium in the megagametophyte during germination supported this possibility during part of the period of germination (fourth to sixth day), but during other intervals the data were without order. The slight increase in ether-soluble magnesium noted after separation from the megagametophytes in seedlings subsequently grown in the dark on LOO's medium is not clearly real and significant. It is of interest that the pattern of accumulation of total ether-soluble magnesium and of chlorophyll in seedlings grown from intact seeds in the dark agrees with that in etiolated barley seedlings upon exposure to light (**19, 20**). The principal difference is in the percentage of the total ether-soluble magnesium accounted for in chlorophyll. SMITH's higher values may result from analysis of leaves only. The entire seedling was analyzed in these experiments. There is a possibility that the megagametophyte provides the seedling with a primary "magnesium acceptor," following the pattern observed by JORDAN and CHIBNALL (**8**) in the embryo of the germinating runner bean. They found that the increase in the amount of the magnesium salt of phosphatidic acid during germination was followed by a replacement with the calcium salt at the onset of chlorophyll synthesis. If, however, this were the case, and if the "acceptance" were reversible and no other step were blocked, then supplying the excised seedling with inorganic magnesium should result in continued chlorophyll synthesis after separation. This did not occur (fig. 9).

The maximum percentage of total ether-soluble magnesium accountable for in chlorophyll was 35.2% in intact seedlings but only 22% in excised seedlings grown in the dark. This may be an indication that steps in chlorophyll synthesis subsequent to the initial incorporation of inorganic magnesium into an organic compound were not proceeding in the excised seedling and may reflect its inability to synthesize other components of the chlorophyll molecule. GRANICK (**5, 6**) has recently isolated iron protoporphyrin 9 and magnesium protoporphyrin from X-ray mutants of *Chlorella*, and he has suggested that the incorporation of magnesium is the step following the production of protoporphyrin in the biosynthesis of chlorophyll. A number of oxidations, reductions, and esterifications must follow the production of the magnesium protoporphyrin and precede the final appearance of chlorophyll. It is highly unlikely, on theoretical grounds, that these final steps occur outside the chloroplast. The continued synthesis of chlorophyll by partially greened excised

seedlings grown in the dark (figs. 4, 9, 10) clearly indicates that, unlike angiosperms, these seedlings can conduct the final steps in the synthesis of chlorophyll in the dark. It follows from this that different, or fewer, steps in the synthesis of chlorophyll are photochemical in *P. jeffreyi* than in angiosperms and that these steps can be carried out without light by the activity of the megagametophyte. Detailed chemical analysis of the ether extracts of seedlings may be profitable.

The temperature coefficient of chlorophyll synthesis in excised seedlings in the dark was found to be 1.69 in the temperature interval 11°–23.5° C. This suggests limiting enzymatic processes. At 34° and 37° C. the rate of synthesis was far below that at 23.5° C. LUBIMENKO and HUBBENET (12) found that a decline in chlorophyll production occurred at temperatures higher than 30° C. in wheat. They suggested the appearance of a new reaction at these temperatures resulting in the destruction of the precursors or their conversion to new colorless products. They obtained temperature coefficients below 1 for the temperature interval 25°–35° C.

During the early phases of germination the rate of chlorophyll synthesis in the dark in partially greened excised seedlings is greater the longer the preceding period of contact between seedling and megagametophyte (fig. 10). Seedlings separated on the tenth day, however, synthesized chlorophyll at a rate slower than those separated on the eighth day. The rate of the reaction may be a function of the amount of available reactants or of enzymes. It would be expected that the older seedlings would contain as much or more enzyme than younger ones, so the lower rate in the 10-day-old seedlings suggests that these differences in rates are, at least, an effect of differences in concentrations of available substrate. Since the number and nature of the enzymatic and chemical steps involved in this synthesis are not known, it does not seem valid to attempt an explanation on the basis of enzyme kinetics.

LUBIMENKO (11) observed that *P. sylvestris* seedlings grown under six sheets of white paper developed about twice as much chlorophyll as those in the dark during the same time interval. The data presented in the present paper show that, at a light intensity of approximately 60 foot-candles, seedlings separated from megagametophytes on the sixth day after the start of germination synthesized chlorophyll at about the same rate as seedlings of the same age in the dark in contact with their megagametophytes (fig. 3; table 2). LUBIMENKO's experiments differed in these respects: (*a*) a different species of pine was used; (*b*) the seeds were under the experimental light conditions from time of germination to harvest, and the seedlings were in contact with their megagametophytes during the entire period; and (*c*) the plants were exposed to light of natural day-length. In addition, LUBIMENKO expressed his results in amount of chlorophyll per unit fresh weight. Thus, the seedlings in LUBIMENKO's experiments were, during at least part of the experiment, under the influence of both their megagametophytes and light. Although there is no evidence that these effects are additive, this may explain the differences. In the present study seedlings separated from megagametophytes at the same time, and grown on the same medium, produced chlorophyll about four times more rapidly in the light than in the dark, during the first 2 days after separation.

The great chlorophyll loss demon-

strated by seedlings placed in the dark after exposure to light and the absence of such loss in seedlings not exposed to light suggests a light-induced, chlorophyll-destroying system.

The difference in rate and extent of chlorophyll synthesis by seedlings placed in light directly after separation and by those kept in darkness for several days prior to exposure is interesting. It may perhaps be profitably examined by a study of the effect of exposure to light at low temperatures in experiments of the type performed by LUBIMENKO (11) and SMITH (20).

Summary

1. Chlorophyll formation in the dark and certain relationships between megagametophyte and sporophyte were studied in germinating seeds and excised seedlings of *Pinus jeffreyi*. Certain aspects of chlorophyll synthesis in light and darkness were also compared.

2. Embryos excised from ungerminated seeds did not form chlorophyll when grown in darkness on various extracts of megagametophytic tissues or on certain synthetic media. The chlorophyll pigments formed in light and darkness were spectroscopically similar. This is a confirmation and extension of some of SCHMIDT's data.

3. Seedlings separated from megagametophytes during the first few days after germination continued to form chlorophyll in the dark for a limited time, but at a slower rate than those remaining in contact with megagametophytes.

4. Seedlings separated from megagametophytes after 6 days in the dark and exposed to light of about 60 foot-candles during the following 2 days synthesized chlorophyll about four times more rapidly than similar seedlings kept in the dark, but at about the same rate as seedlings remaining in contact with their megagametophytes in the dark.

5. The increase in chlorophyll exhibited by seedlings grown in the dark in contact with megagametophytes appears to follow the curves for increase in total magnesium and dry weight. The total magnesium in ungerminated embryos, however, exceeded the amount present in the chlorophyll of the oldest plants examined.

6. Seedlings excised from megagametophytes 6 days after germination in the dark and then grown in LOO's medium in the dark increased in dry weight and total magnesium content, but they synthesized chlorophyll at the same rate and in the same quantity as similar excised seedlings grown on a medium of washed agar and Pyrex-distilled water.

7. Total ether-soluble magnesium in seedlings grown in the dark in contact with their megagametophytes increased during germination, but more slowly than magnesium incorporated in chlorophyll. Four days after the beginning of the experiment 2.5% of the total ether-soluble magnesium was attributed to that in chlorophyll; 6 days later this value was 35.2%. In seedlings separated from megagametophytes 6 days after germination and continued in the dark on LOO's medium the maximum value was 22%.

8. The rate of chlorophyll synthesis and the maximum amount formed in the dark by seedlings separated from megagametophytes and grown in the dark varies with the duration of contact of these two seed components during germination and preceding separation.

9. The temperature coefficient for chlorophyll production in the dark by seedlings separated on the sixth day

after commencement of germination was estimated to be 1.69 in the temperature interval 11°–23.5° C. Chlorophyll accumulation at 34° C. and at 37° C. was considerably below that at 23.5° C.

10. The possibility that different, or fewer, steps in the synthesis of chlorophyll are photochemical in *P. jeffreyi* than have been described for etiolated angiosperm seedlings is discussed.

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ACTION SPECTRUM FOR THE PHOTOPERIODIC CONTROL OF FLORAL INITIATION OF THE LONG-DAY PLANT HYOSCYAMUS NIGER

M. W. PARKER,[1] S. B. HENDRICKS,[2] AND H. A. BORTHWICK[1]

Introduction

Action spectra for the photoperiodic control of flowering have been reported for two short-day plants, Biloxi soybean and *Xanthium* (**4, 5**), and for one long-day plant, Wintex barley (**2**). The results for the three plants were remarkably similar and indicated that the first step in the photoperiodic control of flowering was the same for both long-day and short-day plants. This tentative conclusion was based on the assumption that the result obtained with Wintex barley was general for long-day plants; but the necessity remained of testing this assumption with at least one additional plant. An annual variety of *Hyoscyamus niger* L. was selected because it had been used extensively by other investigators of photoperiodism, seed was available from inbred lines, and the plant differed radically from barley in its mode of growth and development.

Material and methods

It was necessary with *Hyoscyamus*, as it had been with Biloxi soybean, *Xanthium*, and Wintex barley, to perform extensive orientation experiments to establish the conditions under which the action-spectrum studies were subsequently to be done. These orientation experiments were concerned not only with determining suitable light and dark schedules for the spectrograph experiments but also with establishing pre- and post-treatment conditions that would not tend to mask any differences induced by the treatments.

Early experiments with *Hyoscyamus*, grown from seed of a local source, proved too variable for satisfactory results. For all later experiments seed was obtained of inbred lines of *Hyoscyamus* developed by G. MELCHERS and A. LANG. Plants from this seed were grown and mass-pollinated, and the resulting seed was used for all subsequent work.

The seedlings were started in the greenhouse in vermiculite and were transplanted later to 2-inch pots of soil. Many of the experiments were performed with plants in these small pots; others were done with older plants which had been transferred to $3\frac{1}{2}$-inch or 4-inch pots. In either case the plants were subjected to 8-hour photoperiods in the greenhouse until they were used experimentally. This period varied from about 7 weeks to 3 months. Details of treatment and of post-treatment were not identical in all experiments, so they are presented with the individual experiments. Final results were obtained, in most cases 3 weeks after the beginning of the treatment, by dissecting all plants that had not produced visible flower buds. Records of stem length from the lowermost leaf scars to the terminal meristem were made, and the presence or absence of flower buds or primordia was noted.

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Experimentation

Experiments were first performed to test the flowering response of *Hyoscyamus* plants to light-darkness cycles of different length in which the light period was always the same length as the dark period. A cycle thus refers to the total hours of one light and one dark period. Cycles of 24, 12, 8, and 4 hours were tested for a period of 3 weeks. No plants initiated flower buds, nor did any stems elongate on 24-hour cycles, but floral initiation and stem elongation were general on the other three cycles, and no significant differences among those three treatments were evident.

Failure of the 24-hour cycles presumably resulted from the fact that the 12-hour dark periods were too long to promote flowering. The 12-hour light periods were obviously not too short, because cycles containing much shorter light periods were successful. Neither was the total energy supplied during the experimental treatment inadequate, because all four lots received the same total amount. The question was further examined in another experiment which repeated the 24- and 12-hour cycles and included several other schedules intermediate between those in which the light and dark periods were unequal (table 1).

The experiment included three groups of plants which received 12, 15, and 18 days of treatment, respectively. Flower formation was universal except on the plants that received 12-hour dark periods and in one plant that received 11-hour dark periods for only 12 days. Stem lengths were somewhat variable, but among the lots that initiated flowers it is to be noted that the shortest ones were on plants that received 11-hour dark periods. Although 12 days of treatment were enough to induce flower formation on nearly all plants, it is apparent that additional days had a further promotive effect on stem elongation.

Other 24-hour cycles in which the light and dark periods were unequal were then

TABLE 1

EFFECTS OF VARIOUS KINDS OF CYCLES OF LIGHT AND DARK AND DURATIONS OF TREATMENT ON FLOWERING AND STEM ELONGATION OF HYOSCYAMUS

Lot*	Daily hours of light and dark				Plants with flower primordia following various durations of treatment			Stem length following various durations of treatment (cm.)		
	Light	Dark	Light	Dark	12 days	15 days	18 days	12 days	15 days	18 days
1	6	6	6	6	3	3	3	9	19	37
2	7	6	5	6	3	3	3	18	30	40
3	8	6	4	6	3	3	3	14	27	35
4	9	6	3	6	3	3	3	11	25	31
5	10	6	2	6	3	3	3	8	15	31
6	11	6	1	6	3	3	3	4	6	20
7	12	12	0	0	0	0	0	0	0	0
8	6	6	6	6	3	3	3	4	21	35
9	6	7	6	5	3	3	3	10	21	36
10	6	8	6	4	3	3	3	9	18	32
11	6	9	6	3	3	3	3	5	23	35
12	6	10	6	2	3	3	3	2	11	22
13	6	11	6	1	2	3	3	1	6	3
14	12	12	0	0	0	0	0	0	0	0

* Three plants per lot.

tested (table 2). This experiment repeated parts of a similar one by LANG and MELCHERS (3) and was necessary to establish the response that the plants would make under the conditions of the controlled-environment rooms used in this work. The results obtained were essentially the same as theirs. The threshold value for complete effectiveness was found to be a cycle containing about 13 hours of light and 11 hours of dark repeated fourteen or more times.

The difference in effectiveness of cycles containing 11 and 12 hours of darkness was very marked, so it seemed possible that plants receiving 12-hour dark periods could be induced to bloom if the dark periods were interrupted near the

TABLE 2

FLOWERING AND STEM ELONGATION OF HYOSCYAMUS FOLLOWING VARIOUS NUMBERS OF 24-HOUR LIGHT AND DARK CYCLES OF DIFFERENT TYPES

CYCLE (HOURS)		PLANTS FLOWERING IN RESPONSE TO VARIOUS NUMBERS OF 24-HOUR CYCLES*						TOTAL STEM LENGTH PER LOT FOLLOWING VARIOUS NUMBERS OF 24-HOUR CYCLES*					
Light	Dark	4	6	8	10	12	14	4	6	8	10	12	14
10	14	.....	.....	.....	.....	.....	0	.....	.....	.....	.....	.....	0
11	13	.....	.....	.....	0	0	0	.....	.....	.....	0	0	0
12	12	.....	.....	.....	0	0	0	.....	.....	.....	0	0	0
13	11	.....	.....	.....	1	3	6	.....	.....	.....	7	14	20
14	10	.....	.....	3	6	6	.....	.....	.....	23	74	56	.....
15	9	.....	3	6	6	.....	.....	.....	20	67	176	.....	.....
16	8	.....	5	6	6	.....	.....	.....	38	110	280	.....	.....
17	7	0	6	6	.....	.....	.....	6	37	106	.....	.....	.....
18	6	6	6	6	.....	.....	.....	17	37	102	.....	.....	.....

* Values based on lots of six plants. All lots were returned to 10-hour photoperiod following the last cycle, and dissections were made 21 days after the start of treatment.

TABLE 3

FLOWERING AND STEM ELONGATION OF HYOSCYAMUS ON 24-HOUR CYCLES CONTAINING PHOTOPERIODS OF 12½, 12, AND 11½ HOURS AND WITH THE RESPECTIVE DARK PERIODS INTERRUPTED NEAR THE MIDDLE WITH 1 HOUR OF RADIATION AT VARIOUS ENERGIES

ENERGY APPLIED DURING 1-HOUR DARK-PERIOD INTERRUPTION (FT-C.-MIN.)	NUMBER OF PLANTS WITH FLOWER BUDS AFTER TREATMENT AT DIFFERENT PHOTOPERIODS*			LENGTH OF STEMS AFTER TREATMENT AT DIFFERENT PHOTOPERIODS*		
	12½ hr.	12 hr.	11½ hr.	12½ hr.	12 hr.	11½ hr.
6000	3	3	3	50	60	48
3000	3	3	2	75	49	26
1500	3	3	1	75	33	8
750	3	3	2	103	49	22
375	3	0	1	87	10	8
187	2	2	0	38	12	6
94	0	0	0	c	c	0
47	0	0	0	0	0	0
24	1	0	0	9	0	0

* Based on lots of three plants each. Photoperiodic treatments were applied for 12 days after which all lots were subjected to 10-hour photoperiods and uninterrupted 14-hour dark periods. Plants were dissected 21 days after start of photoperiodic treatment.

middle with a small amount of radiant energy. Threshold experiments were accordingly run with 24-hour cycles containing $12\frac{1}{2}$, 12, and $11\frac{1}{2}$ hours of light and dark periods of $11\frac{1}{2}$, 12, and $12\frac{1}{2}$ hours inclusive of the interruption. Near the middle of each dark period, radiant energy from an incandescent-filament lamp was applied at various intensities for 1 hour (table 3). The energy was 6000 foot-candle-minutes at the first station of the threshold, and it decreased by a factor of two through nine stations. The responsiveness of the plants to comparatively small amounts of energy was clearly evident, and the effectiveness of a given amount of energy applied in the middle of the dark period decreased as the length of the dark period, inclusive of the interruption, increased.

The various experiments thus far presented were performed with plants that had established large rosettes in 4-inch pots. Earlier experiments had indicated that smaller plants in 2-inch pots might prove equally responsive, and, since their small size would better suit them to the requirements of the spectrograph, a threshhold experiment was performed with plants of that type. Flowering in this experiment was induced in all plants that received 94 foot-candle-minutes or more of radiant energy during the dark-period interruption (table 4). This was a value which previous experience with other plants had demonstrated to be low enough to make satisfactory spectrograph experiments possible. An abrupt increase in length of stem occurred at the lowest energy at which flowering occurred.

It was necessary to perform one further experiment preliminary to the experiments with the spectrograph, to determine the part of the dark period during which the effect of a given amount of radiation would not deviate significantly from the maximum effect. For such a study four separate threshold experiments were performed simultaneously. The plants all received 24-hour cycles containing 12-hour periods of high-intensity radiation. Beginning 6, 7, 8, and 9 hours, respectively, after the start of the dark periods, the various lots were subjected to 1-hour dark-period interruptions at nine different intensities starting at 100 foot-candles and decreasing by a factor of 2 at each successive station. The treatments at highest intensities were effective in all four series, and the sharp break from effectiveness to noneffectiveness in this experiment occurred between energies of 375 and 187 foot-candle-minutes in each series. This indicated that reasonably uniform results could be expected if the dark-period interruptions occurred within the 3-hour period beginning 6 hours after the start of the dark period.

TABLE 4

ENERGY REQUIRED DURING 1-HOUR INTERRUPTION OF DARK PERIODS TO PROMOTE FLOWERING AND STEM ELONGATION OF HYOSCYAMUS PLANTS RECEIVING DAILY 12-HOUR PHOTOPERIODS

Energy applied during 1-hour dark-period interruptions* (ft-c.-min.)	Total plants flowering per lot†	Mean length of stems per plant (mm.)
6000	9	140
3000	9	149
1500	9	130
750	9	102
375	9	85
187	9	47
94	9	20
47	5	8
24	1	4
12	0	4
6	0	4
Uninterrupted control	0	3

* Unfiltered radiation from 500-watt incandescent-filament lamp applied during eighteen successive daily interruptions.

† Lots of nine plants each subjected to 10-hour photoperiods following 18 days of treatment. Dissected 27 days after start of treatment.

The threshold experiments described above, which gave the data required for designing the spectrograph experiments, involved brief interruptions of the dark period. The following threshold experiment, which was of general interest in connection with the response of *Hyoscyamus* to light but of secondary importance in planning the spectrograph experiments, was one in which the dark periods were completely replaced by different amounts of radiation. This experiment was performed in the greenhouse and adjacent "dark houses." The plants received natural radiation from 8:00 A.M. to 4:00 P.M. daily between the dates of June 9 and July 20. From 4:00 P.M. to 8:00 A.M. they were placed in a "dark house" where the nine different lots received continuous radiation at intensities that decreased by a factor of 3 from 60 foot-candles to about 0.01 foot-candles. The plants used were vigorous rosettes growing in 4-inch pots. During the 41-day period of the experiment, flowering occurred in several of the lots. It took place within a few weeks at the three stations of highest energy, somewhat more slowly at the fourth station, and at the fifth station stem elongation was just starting at the end of the experiment. Plants at the remaining stations were dissected at that time; flower primordia were present on the three plants of the sixth station, but they were not present on those of the remaining stations. Intensity at station 6 was 0.25 foot-candle; at station 7, 0.08 foot-candle. Flowering thus occurred when 240 foot-candle-minutes of energy were added but failed with approximately 80 foot-candle-minutes. The experiment is of interest in showing that flowering can be induced under conditions quite different from those described in earlier experiments and with not significantly greater energy.

During the experiments with *Hyoscyamus* it seemed that the plants were showing a hyponastic response in a single day to a stimulation that would induce flowering if repeated for 10 days. It was thought that this effect was rather tenuous, since hyponasty also resulted from changes accompanying transfer of plants from the greenhouse to growth room. That the effect was real was rather strikingly shown in a "threshold" type experiment in which lots of *Hyoscyamus* plants were placed throughout their dark periods at various distances from an incandescent light. A decrease of hyponastic response was correlated with decrease of light intensity through the region of intensities required for floral initiation.

SPECTROGRAPH EXPERIMENTS

The spectrograph, the light source, and their appurtenances were the same as previously described in the work on the action curves for floral initiation in soybean, cocklebur, and barley (**2**, **4**, **5**). The pots containing the plants were placed horizontally in the light path with the rosette facing the light source. Since it was found that response was not critically dependent on size of plant, small plants in 2-inch pots were used because they intercepted narrower wave bands in the spectrum and because greater numbers could be included in a single treatment. To facilitate handling, these pots were placed in small rectangular boxes in lots of eight or more. The spaces between the pots and the box were filled with vermiculite, which kept the pots from drying too rapidly. In experiments concerned with the red end of the spectrum the slit width was about 4 mm. At the plant position this represented about 1 inch, or half the pot diameter, which gave maximum incident energy for the resolving power imposed by the plant

size. Intensities were increased in experiments designed primarily for the blue end of the spectrum by increasing the slit to about 12 mm., which in effect utilized the entire crater of the arc as a source.

Different energies in a particular wave-length region were obtained by varying time of exposure at constant intensity. This, of course, was based upon the assumption that a given amount of energy produced the same response irrespective of intensity, i.e., that reciprocity held for the time intervals used. No direct test was made of this assumption for *Hyoscyamus*, but it had been demonstrated to be valid for soybean, cocklebur, and barley.

Results of value were obtained from eight spectrographic experiments. One of the most successful of these was done with the narrow slit. It was designed to give detailed information concerning the red end of the spectrum, but it covered the entire visible region (fig. 1). This experiment included eighteen stations between 4130 A and 5400 A at which single plants were irradiated 101 minutes. The plants at stations between 4130 A and 4950 A, not shown in figure 1, were all vegetative upon dissection. There were seven stations in the region from 5610 A to 6890 A. Times of irradiation for these stations were 1′00″, 2′31″, 6′21″, and 16′, or a total of about 26 minutes, thus permitting four replicates at each energy level at each station within a total elapsed time of less than 2 hours. At one further station, placed at 7310 A, two plants were irradiated 50 minutes each.

The sloping lines of figure 1 indicate the energies obtained through different durations of irradiation. The numbers above these lines show the mean length of stems in millimeters, and the figures below give the fractional number of plants in a given treatment that formed flower primordia; that is, a value of 0.75 indicates that three of the four plants in a lot formed flower primordia.

A response curve was drawn through the points on the lines of energy value where a sharp break in biological effectiveness occurred. The action curve between 5500 A and 5100 A was plotted on a different energy scale at the left of figure 1, to show a point corresponding to 101 minutes of irradiation.

For another experiment the broad slit on the spectrograph was used in order to give as great energy in the blue portion of the spectrum as possible (fig. 2). The resolving power in the red part of the spectrum thus was reduced but still was adequate because the action curve was relatively flat in the red as was shown by the experiment described above and by others in which the narrow slit was used. For this experiment plants were irradiated at nineteen wave-length stations from 4000 A to 6900 A. The durations of irradiation and therefore the numbers of plants that could be irradiated differed in different parts of the spectrum. At each of twelve stations between 4000 A and 4470 A two plants were irradiated 96 minutes. At 5500 A two plants each were irradiated 2, 4, 8, 16, and 32 minutes, respectively. At four other stations between 5750 A and 6900 A four plants were irradiated for each of these five time intervals. In order that the two 96-minute irradiations might be applied at the same relative time in the dark period, the experiment was conducted in two controlled-environment rooms. At stations between 4000 A and 4400 A not more than one plant in any lot of two formed flower primordia, so these points were not shown in figure 2. The action curve was indicated as rising to higher energy values at wave lengths longer

than 4470 A, but no experimental points at energies lower than those corresponding to 96 minutes were obtained between 4500 A and 5160 A. If points in this region had been determined, it is probable that an energy requirement between 1500 and 3500 kiloergs/cm.^{2} would have been found. This assumption was based mainly on observations of stem lengths of lots irradiated 96 minutes.

Another experiment, run with the wide slit in the spectrograph, was very similar to the one just described except that plants at stations between 4000 A and 5160 A received 60 minutes instead of 96 minutes of irradiation. In the red

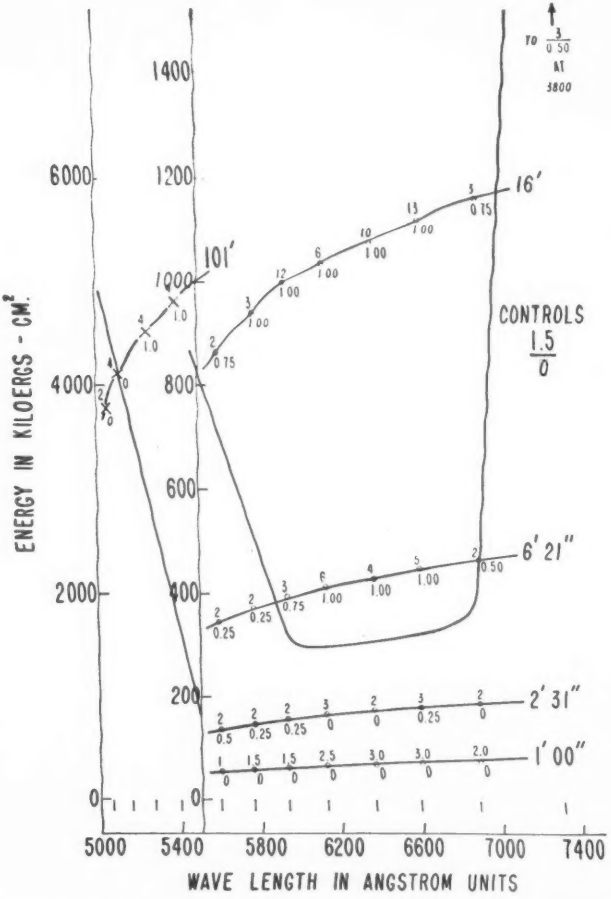

FIG. 1.—Response curve for initiation of flowers and elongation of stems by *Hyoscyamus* in range 5100 A to 7310 A. Energies resulting from irradiation times of 1 minute to 101 minutes are shown in five curves. Region from 5100 A to 5500 A is plotted on different energy scale at left. Paired numbers at positions along these curves corresponding to various wave-length stations give mean stem length (upper number) in mm. and fractional number of plants that initiated flower primordia at given station (lower number); i.e., value of 1.00 indicates that all plants of given lot flowered. Response curve was drawn freehand through energy lines, crossing them between points at which sharp break in response occurred.

end of the spectrum the plants flowered in response to 2-minute periods of irradiation as they did in the experiment of figure 2. The plants of the two experiments were thus about equally sensitive to identical treatments in the red, but the ones in the blue end of this experiment failed to flower with 60-minute treatments. On the basis of the combined results of the two experiments one would infer that between 4400 A and 5160 A the action curve was approximately parallel to the 96- and 60-minute energy lines and about midway between them. A point on an action curve was established at 5200 A for this experiment at an energy approximately 45-fold greater than at 6200 A. Although the results of the two experiments fairly well define the position of the action curve in the blue region, a curve was not actually drawn through the region, because the

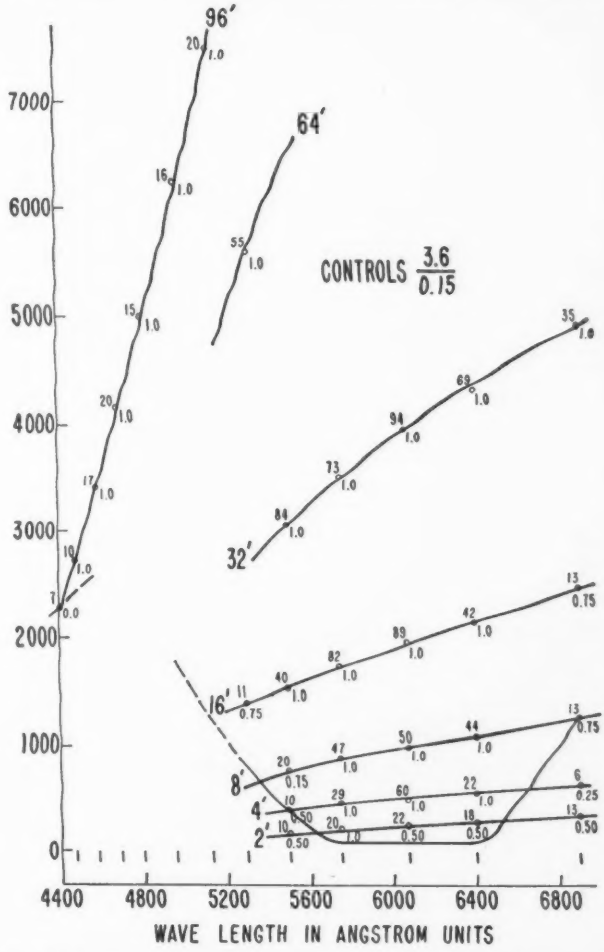

FIG. 2.—Results from another experiment with *Hyoscyamus* for region from 4400A to 6900 A

necessary data were not available from any single experiment. Results of two other experiments for the blue portion of the spectrum were not essentially different from those described above.

All eight successful spectrograph experiments gave results in the red that indicated the same relative change in effect with wave length; but the absolute energies required to cause flower-bud formation at a given wave length varied among experiments by as much as tenfold. Values from the experiment of figure 1 were among the lowest observed. This variation was largely the result of differences among lots of plants, for the experiments were performed at various seasons of the year with plants that were grown in the greenhouse. Within a given experiment, however, the plants were uniform in their floral response. The energy required to induce stem elongation was readily established to be the same as that for floral initiation; but the variation of length with energy above the minimum was not determined.

Discussion

The action curve for floral initiation of *Hyoscyamus*, representing the average of

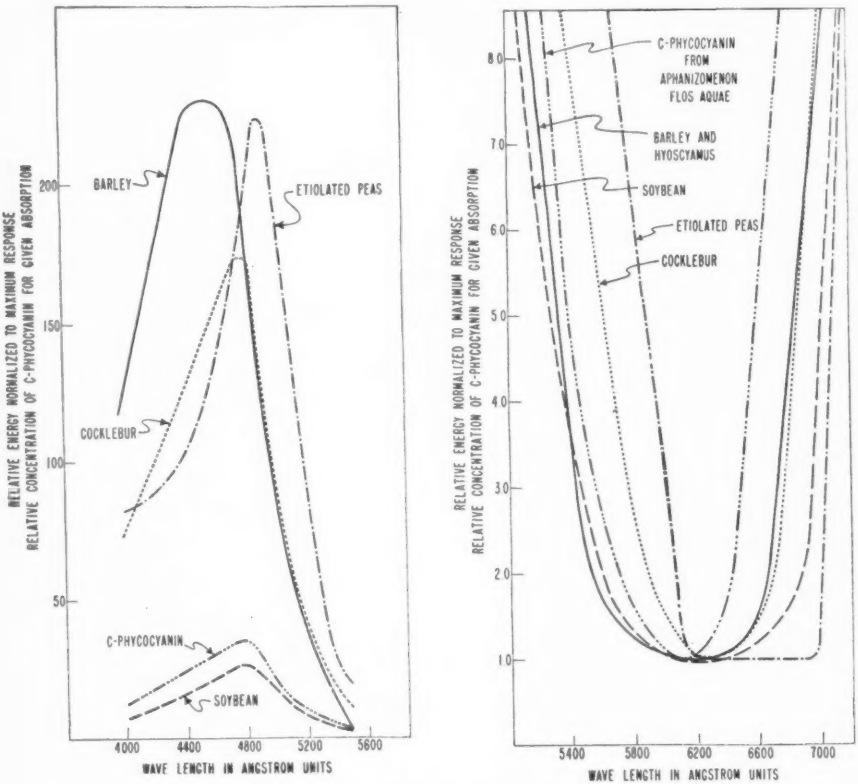

FIG. 3.—Composite action curves for control of floral initiation of several long- and short-day plants and for regulation of leaf size in etiolated peas, together with curve showing relative concentrations of *c*-phycocyanin for a given absorption.

the various experiments for the red portion of the spectrum, is shown in figure 3 in comparison with those of other short- and long-day plants and for leaf lengthening of etiolated pea seedlings (**1, 6**). The several curves are closely similar, and no great emphasis should be placed on the differences that can be noted on the figures. The differences are experimentally valid and considerably exceed reasonable limits, but it should be borne in mind that the response criterion is one of complex growth in which the operation of the initial causative phenomenon is only a determinative factor subject to many later modifying actions. More specifically, each experimental plant was brought under optimum conditions for experimental treatment such that interruption of a succession of dark periods with radiation from the spectrograph produced a measurable plant response. This meant very careful choice of daylength, duration of treatment, age of plant, and other factors.

Although the action curve for floral initiation in *Hyoscyamus* was not completely determined in the blue, it probably was closer to that for barley than for soybean (fig. 3). The variation of relative energies required for floral initiation in the region of minimum sensitivity near 4800 A was greatest between soybean and the other three plants. Again, however, this could well have arisen from the factors mentioned above. In the end the differences might lead to interesting deductions, but at this stage they should not obscure the fundamental similarity of response.

A curve showing the relative concentrations of phycocyanin from the blue-green alga *Aphanizomenon flos-aquae*, as calculated from the data of T. SVEDBERG and T. KATSURAI (**7**), required for a given absorption, is also shown on the figures. The striking similarity of its absorption to the action curves is apparent. Much could be said on this point, but certain cautions should first be stated. Discussion of open-chain tetrapyrrole type pigments of which *c*-phycocyanin is representative does not make any less probable a porphyrin type pigment, such as protochlorophyll, with its absorption modified by screening pigments (**1**). These two classes of pigment differ in that the latter has a much higher absorption in the blue portion of the spectrum than in the red, while the former has a low absorption in the blue. Differentiation between the two possibilities must await results of studies on response of albino plants.

Summary

1. Vegetative plants of *Hyoscyamus niger* can be induced to flower by application of relatively small amounts of radiant energy near the middle of dark periods that would prevent flowering if uninterrupted.

2. Interruptions of 12-hour dark periods with energies corresponding to about 100 foot-candle-minutes of light were effective in causing initiation of flower primordia. Minimum energy to cause a given effect was relatively constant if the interruption was made during the 2-hour period following the middle of the dark period.

3. The action curve for floral initiation of *H. niger* was readily established in the region from 7300 A to 5600 A. Limitation of energy from 5600 A to 4000 A made it impossible to establish a continuous response curve for this region, but sufficient points were obtained to establish the limits of effectiveness. This curve is essentially the same as those for

control of flowering in soybean, cocklebur, and barley, and for regulation of leaf size in etiolated peas.

4. Similarity of the action curve for control of initiation of flower primordia in *Hyoscyamus* to the absorption of phycocyanin was noted, and the possibility that the effective pigment for the photoperiodic reaction might be some type of straight-chain tetrapyrrole was pointed out.

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PERFORATION OF THE SURFACE MEMBRANES OF NUCLEI AND PLASTIDS BY NUCLEODESMATA AND PLASTODESMATA

FLORA MURRAY SCOTT

Introduction

The protoplasts of individual cells are interconnected by plasmodesmata, so that living protoplasm forms a continuous network throughout the plant. It has long been known that cells when first mounted and examined microscopically are in general optically clear except for cell wall, chloroplasts, and nucleoli and that the details of internal structure which appear later result from localized changes in the hydration of the protoplasm. Although each protoplast is considered physiologically as a constantly changing, complex, colloidal system, graphically it is consistently but necessarily represented in its most clearly visible condition of partial dehydration, a network of cytoplasm surrounding a reticulate nucleus. The principal strands of cytoplasm are anchored in plasmodesmatal pits in the cell wall. The nuclear net is separated from the cytoplasmic, except during nuclear division, by a continuous nuclear membrane. Within the cytoplasm itself the stroma of the plastids is similarly shown as completely isolated by an imperforate limiting surface.

In this paper it will be shown that the membranes surrounding both nuclei and plastids are in reality perforate envelopes. The reticulum of the nucleus is connected with that of the cytoplasm by nucleocytoplasmic fibrillae, "nucleodes-

mata"—a term suggested by my colleague, Dr. KARL HAMNER. Similar fibrils, "plastodesmata," extend outward from the plastid stroma, traverse the limiting membrane, and unite with the surrounding cytoplasm. Thus, within each cell, nucleus and cytoplasm together constitute a unified protoplasmic network, an integral part of the continuous network of the plant as a whole.

It appears probable that nucleodesmata and plastodesmata may resemble the artifact spindle fibers of mitosis and that they may therefore be regarded as microcanals which, in the fully hydrated, actively metabolic cell, function in the transport of materials. It is therefore recognized that the terms "nucleodesmata" and "plastodesmata," coined after the parent term "plasmodesmata," although apparently structurally accurate, may be misleading from the physiological standpoint.

Material and methods

The nuclei of the endosperm and nucellus of *Echinocystis macrocarpa* are magnificent objects for nuclear study, since they may measure up to 200 μ in diameter (**16, 17**). The best time for collecting material may be gauged more or less accurately by the condition of the spiny fruit. When the fruit has reached its full size, and the spines are beginning to harden, the seeds are also at maximum size and the seed coats still soft. Within the seed the endosperm is replacing the nucellus, and the embryo is developing rapidly. When the cotyledons measure about one-third the length of the seed, endosperm and nucellar cells and nuclei reach their maximum volume. All cells are extremely turgid, and, when cut or punctured, their nuclei are released in the fluid cytoplasm and therefore require no additional mounting medium. Nuclei may thus be examined either within, or free from, the cells.

Phase, dark-field, and polarizing microscopes were used and served to corroborate, or to clarify, the observations made under ordinary illumination.

Of the microchemical methods used, IKI-H_2SO_4, a test for cellulose, as previously used for plasmodesmata (**18**), proved to be most generally useful, though unfortunately temporary. During the initial fixation in IKI some shrinkage occurs, but all protoplasmic components are clearly stained. Careful irrigation with H_2SO_4 temporarily accentuates and permanently darkens nuclear and cytoplasmic networks.

For semipermanent preparations cotton blue in lactophenol causes almost no shrinkage and is consequently useful as a mounting medium. Other reagents and stains which proved more or less useful include osmic acid, Sudan III, Feulgen's reagent, toluidin blue, anilin blue, and ruthenium red.

Interesting preparations are obtained by staining in toluidin blue or some other aqueous stain and thereafter allowing the nuclei to dry out (3–12 hours). After dehydration the shrunken nuclei are cleared rapidly in xylol and mounted immediately in Canada balsam.

Somatic material for comparison with the endosperm nuclei of *Echinocystis* consisted of epidermal and subepidermal tissues of leaves, petioles, and stems of *Acanthus mollis* and *Tropaeolum nasturtium*, with the methods the same as for *Echinocystis*.

For the opportunity to examine additional material I am deeply indebted to my colleague, Dr. BALD (**1, 2, 3**). In his recent virus studies epidermal tissues of healthy and infected *Nicotiana* and other plants were fixed and stained by many different methods. The variation in the

preparations thus obtained proved to be of the utmost value for cytological study. The occurrence of nucleodesmata and plastodesmata was confirmed in all the slides examined by me.

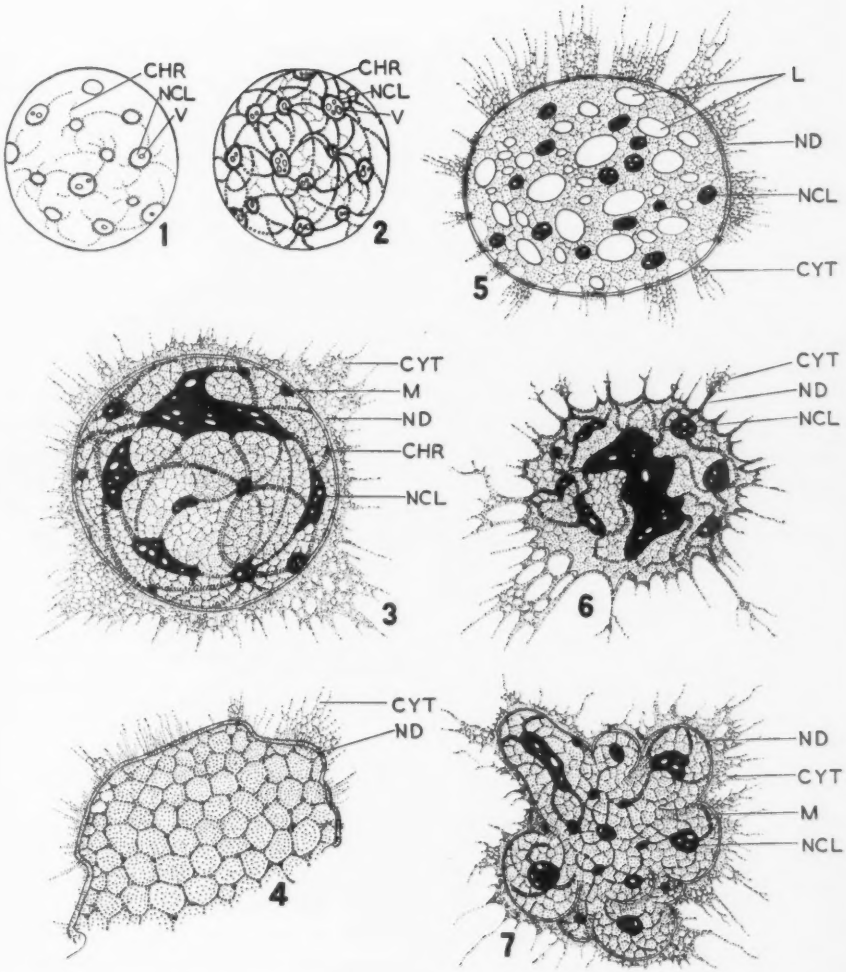
Observations

E. MACROCARPA

When first mounted the nuclei of the endosperm and the nucellus, whether within the cells or floating free in the cytoplasm, are but faintly visible and are optically homogeneous except for the numerous refringent nucleoli. Within a few minutes of mounting, 3–10 or longer, the reticular structure of the nucleus gradually becomes defined, and at the same time the nucleus shrinks more or less in volume (figs. 1, 2, 3, 13). It is then possible to distinguish the details of nuclear structure and to observe that the nucleus is moored in place within the cell by tenuous strands, nucleodesmata, which traverse the nuclear membrane and connect reticulate nucleoplasm with reticulate cytoplasm. The direction of the nucleodesmata on both the outer and the inner surfaces of the nuclear membrane is radial for a distance of about 1 μ. The term "nucleodesmata" indicates the resemblance of these fibrillae to the ubiquitous intercellular plasmodesmata and at the same time serves to differentiate their position. Nucleodesmata are in general extremely slender, actually at the limit of microscopic visibility. Here and there, with an occasional semblance of repetitive pattern, slightly thicker strands may occur. It is considered that such strands result from more dense groupings of the fibrillae, possibly, but not necessarily, artifact.

The nuclear membrane is a well-defined transparent envelope about 0.5 μ thick and appears anisotropic under polarized light. When the nucleus is damaged, the membrane may become wrinkled (fig. 15). In tenuous membranes such as this microchemical tests are inconclusive, but the lipoproteic structure considered probable (**5, 19**) is indicated by faint staining with Sudan III and by clear coloration with IKI. When membranes are torn during the inescapable cutting and puncturing of some cells preparatory to mounting, they remain intact and do not cytolyze. In all cases observed the damaged nuclei immediately coagulate. Strips and flaps of nuclear membrane which are torn free may be examined from the inner or outer surface and also in profile at the point of junction with the rest of the envelope, as well as in optical section in intact nuclei (fig. 4). The markings of the membrane correspond to the distribution of the nucleodesmata. In surface view the membrane is finely punctate, and in profile both inner and outer surfaces are minutely scalloped (fig. 9). The surface pitting, the profile indentations, and the radial striae mark the position of the nucleodesmatal canals.

Under varied conditions so far not clearly defined, lacunae develop within the nuclear membrane (fig. 5). When first observed these pores may be few in number and relatively small (3–5 μ in diameter). They may remain so or may enlarge more or less rapidly in area and may also increase markedly in number until the membrane appears spongelike in surface view. Formation of lacunae may occur when the nuclei are mounted in cytoplasm only, or it may take place in sections irrigated with IKI and occasionally with other reagents. Since IKI causes protoplasmic shrinkage, it is possible that in this case pore formation results from localized contraction of the protein components of the karyotheca,



FIGS. 1–7.—*Echinocystis macrocarpa* endosperm nucleus. *CHR*, chromatin; *CYT*, cytoplasm; *L* lacuna; *M*, membrane; *NCL*, nucleolus; *ND*, nucleodesmata; *V*, vacuole. Fig. 1, shortly after mounting, nucleoli visible, chromatin faintly indicated. Fig. 2, same nucleus as in figure 1 after chromatin reticulum is defined. Fig. 3, with major and minor chromatin nets and nucleodesmata. Fig. 4, part of nuclear membrane seen from inner surface. Fig. 5, during formation of lacunae in membrane. Fig. 6, nucleus desiccated on slide. Fig. 7, in lobed condition.

from shrinkage of the underlying chromatin reticulum, or from both these factors.

On the outer surface of the nuclear membrane and connected thereto by nucleodesmata lies the cytoplasmic sheath, and on the inner surface, similarly attached, the chromatin reticulum (figs. 3, 13, 14). The surface reticulum of chromatin incloses the karyolymph. At this stage in development the pattern of the chromatin resembles that of early prophase rather than that of a typical metabolic or energic nucleus (19). To facilitate description, the chromatin may be considered as consisting of a major and a minor network. The chromonemata of the major net are conspicuous and co nparatively wide (2–3 μ in diameter) but vary in thickness from strand to strand and may also vary throughout the length of the individual strand. The outlines of the meshes are erratic as are those of the nucleoli from which they appear to radiate. All chromonemata are finely granular throughout, the granules similar in size and not more than 1 μ in diameter. The increased width of the major strands appears to be a result of multiple chromonemata, i.e., to polyploidy (fig. 3).

The minor network fills the entire area between the larger strands and, unlike the major, is comparatively regular in pattern. The meshes are polygonal and consistently small (5–10 μ in diameter), and the bounding chromonemata uniformly single. Under dark field certain small but brilliant points of light in a somewhat repetitive pattern may indicate the presence of chromocenters in this minor net. In all preparations examined the mesh areas appear finely punctate, the punctate pattern corresponding to the pitting of the nuclear membrane. It thus appears that nucleus and cytoplasm are interconnected by fibrillae which project from the nuclear surface and traverse the nuclear membrane. In the curious shrunken nuclei produced by dehydration, the connection of nucleoplasm and cytoplasm is again evident (fig. 6). Where the nuclear membrane is not concavely indented, the projecting nucleodesmata remain connected with the cytoplasm.

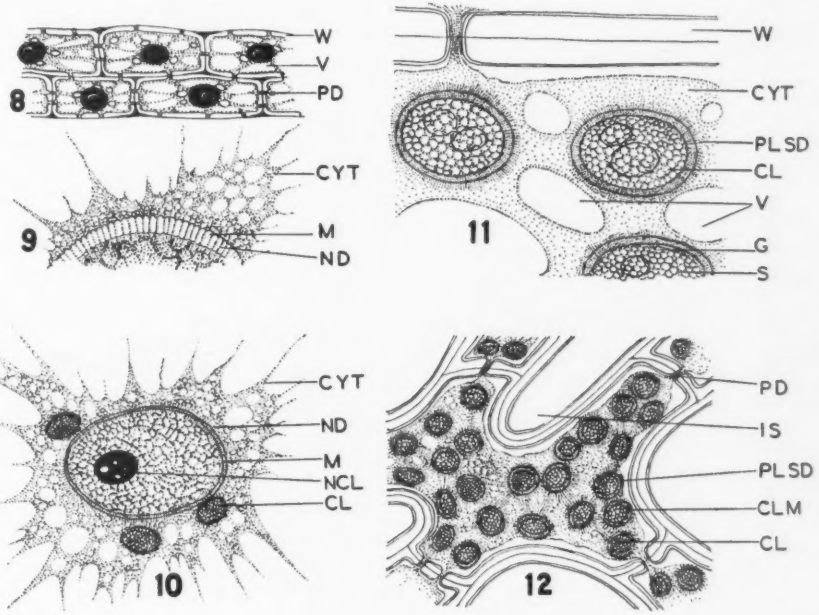
Prior to complete digestion of endosperm and nucellus the outline of the nuclei may change from spherical or ellipsoidal to a lobed condition (fig. 7). The nucleoli generally become segregated, and one nucleolus, or rarely more than one, migrates into each lobe. Although multinucleate cells have so far not been observed at this time, occasional small mononucleolate nuclei may occur floating free in the cytoplasm. It is possible that lobing represents an initial stage in amitotic division. The latter may be completed only on the ultimate breakdown of the cell walls. At all stages of lobation nucleodesmata are observed over the entire nuclear surface and are in no way distinguishable from those of the younger nuclei.

T. NASTURTIUM AND A. MOLLIS

Since the nuclei of *Echinocystis* are unique in size and specialized in physiological condition, it is necessary to examine more "normal," i.e., somatic, cells in order to determine the prevalence of nucleodesmata. In the epidermal and underlying cells of *A. mollis* and *T. nasturtium* the nucleus is more or less central in the vacuolate cells (fig. 8). As a rule, one nucleolus only is present. Chloroplasts are numerous in guard cells, few in the rest of the epidermis, abundant in leaf mesophyll, and less numerous in the subepidermal tissue of the petiole. A few leucoplasts occur in the epidermis of both species.

As in *Echinocystis*, nucleodesmata interconnect nucleoplasm and cytoplasm in *Acanthus* and in *Tropaeolum*. The fibrillae project from the entire nuclear surface and, although at the limit of visibility, are nevertheless clearly evident (figs. 9, 10).

the nuclei. By the time that the nuclear membrane and nucleodesmata are clearly stained with IKI, the surface membranes of the chloroplasts also stand out distinctly, and the internal structure of stroma, grana, and starch grains is equally evident (figs. 11, 12). From the surface



FIGS. 8–12.—*CL*, chloroplast; *CLM*, chloroplast membrane; *CYT*, cytoplasm; *G*, granum; *IS*, intercellular space; *M*, membrane; *NCL*, nucleolus; *ND*, nucleodesmata; *PD*, plasmodesmata; *PLSD*, plastodesmata; *S*, stroma; *V*, vacuole; *W*, cell wall. Figs. 8–11, *Tropaeolum nasturtium*. Fig. 8, epidermal cells. Fig. 9, part of nuclear membrane in epidermal cell. Fig. 10, nucleus of epidermal cell. Fig. 11, subepidermal cells of petiole. Fig. 12, *Acanthus mollis*. Mesophyll cell with chloroplasts; protoplast partially shrunken with IKI-H_2SO_4.

While observing the behavior of the nucleus, attention was naturally directed to the chloroplasts, conspicuous and numerous in many of the cells. Chloroplasts, whether circumnuclear as in younger cells or peripheral as in older ones, are invariably closely surrounded by cytoplasm. The microchemical reactions of the chloroplasts in both *Acanthus* and *Tropaeolum* keep pace with those of

of the stroma there project fibrillae, plastodesmata, which traverse the plastid membrane and unite with the cytoplasmic net. Plastodesmata, like nucleodesmata, are consistently radially oriented, and the radial direction is evident for a distance of about 1 μ on both the outer and the inner surfaces of the limiting layer. Like nucleodesmata, plastodesmata also are at the limit of visibility.

NICOTIANA

In addition to my own preparations, the excellent slides of Dr. BALD of healthy and virus-infected plants proved to be of the utmost value. The epidermal tissues of *Nicotiana* and other forms, fixed and stained by numerous and varied techniques, provided a wide range of material most valuable for the detailed cytological study of nuclei and chloroplasts (**1, 2, 3**). In all the slides examined by me, nucleodesmata and plastodesmata appeared more or less clearly stained. As in my own preparations, fibrillae extend from the surface of the nuclear reticulum or from the stroma of the plastid, traverse the limiting membranes, and unite with the cytoplasmic reticulum. The fibrillae also are radially oriented, and this radial direction continues for about 1 μ beyond the nuclear surface. Again, as in my own preparations, nucleodesmata and plastodesmata are tenuous and at the limit of visibility, and the wider strands which are occasionally observed are considered as localized groupings, natural or artifact, of individual fibrils.

FIG. 13.—*Echinocystis macrocarpa* endosperm nucleus free from cell; fixed in osmic acid, showing connection of nucleus and cytoplasm by nucleodesmata through nuclear membrane.

Discussion

When a nucleus is first distinguishable within a living cell, it is optically homogeneous except for nucleoli. Only after a certain degree of shrinkage in volume, presumably resulting from dehydration, can any internal structural pattern be observed. Contraction in volume similar to that seen in *Echinocystis* (**16, 17**), *Acanthus*, and *Tropaeolum* is readily demonstrable in the epidermis of *Ornithogalum* and similar monocotyledons where the parallel walls of the rectangular cells provide a natural scale of reference during observation. In *Ornithogalum* when the nucleus is first faintly visible within the cell, its diameter appears barely 2–4 μ less than that of the cell. By the time that the nuclear reticulation is clearly defined, the surface of the nucleus is several microns distant from the cell walls, the diameter may have decreased from 25 to 50% in length, and the volume has diminished proportionally. It is therefore evident that nuclear volume (**21**) as hitherto recorded gives an entirely erroneous picture of the nucleus in an undisturbed, normally active cell.

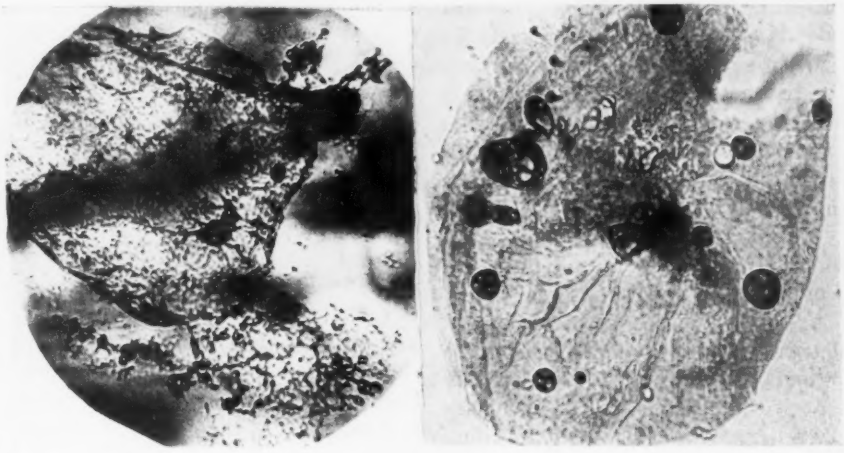
Although the structure of the nucleus has been the subject of a vast amount of recent work (**8, 13**) "from many points of view and by very diverse techniques," in all these varied studies, so far as I am aware, the nuclear membrane is calmly taken for granted, and detailed investigations of its development and function are curiously lacking (**11**). In meristematic cells during division, nucleoplasm and cytoplasm are no longer separated by a visible membrane, and ergastic substances are therefore presumably free to move throughout the entire cell. During

the reconstruction of the nucleus in telophase the nuclear membrane re-forms, probably as a monomolecular layer in its earliest stages. The limiting membrane of the mature nucleus is a finely reticulate perforate envelope, presumably lipoproteic in nature and crystalline in submicroscopic pattern. The origin of this membrane is a problem awaiting solution. It is possible that, like nucleoli, it may result from the activities of specific regions of the chromonemata. On the other hand, intracellular membranes, whether inclosing nuclei, plastids, mitochondria, vacuoles, or the actual surface of the protoplast, may be more or less uniform throughout the cell, rather than specific, in which case chromonematal action would play no part in their construction. The occasional use of the term "precipitation membrane" (**8**) without definitive details does not appear to clarify the problem.

In *Echinocystis* prior to amitotic division and ultimate disintegration and absorption, the endosperm nuclei pass through a characteristic lobed condition. Intensive studies by means of X-rays, polarized light, and also the electron microscope will be required to trace the changes in the submicroscopic structure of the nuclear membrane during this marked increase in surface area.

Nucleodesmata, to judge by their staining both in my own preparations and in those of Dr. BALD, appear to be fibrillar extensions of chromatin rather than of karyolymph. Individual fibrillae are at the limit of visibility, and the intermittent heavier strands, almost 1 μ in diameter, presumably represent grouped fibrillae, the groupings possibly artifact. The heavier strands appear to be connected with the wider chromonemata, the former with the minor chromatin network.

Nucleodesmata are distinguishable in fresh material under the phase microscope and appear there, as elsewhere, as fine protoplasmic threads. The fibrillar



FIGS. 14–15.—*Echinocystis macrocarpa* endosperm nucleus within cell, treated with IKI-H_2SO_4. Fig. 14 (*left*), showing nucleocytoplasmic connection. Fig. 15 (*right*), crushed, showing wrinkling of nuclear membrane.

appearance is intensified by fixation and staining with IKI or other reagents. The question may be raised as to whether nucleodesmata are not in reality tubules, microcanals, or canaliculi. In stained preparations of meristem, spindle fibers appear as slender threads (**10, 19**). In living cells of the same tissue, the spindle is differentiated into semigelatinous matrix and longitudinal canals in the fluid of which Brownian movement may occasionally be observed. Whether or not nucleodesmata are in fact similar to spindle canals, undoubtedly when fully hydrated they are well able to function in the exchange of materials between nucleus and cytoplasm, evidence of which appears in the intracytoplasmic nucleoproteins recently demonstrated by CASPERSSON and his colleagues (**6, 7**). They may also be considered significant in future discussions of plasmagenes.

The position of the nucleus within any cell varies somewhat according to cell type and cell age. Thus, in meristem cells the nucleus is central, while in vacuolated cells it is central or parietal. The nucleus of a developing root or shoot hair lies close to the tip of the papilla and is carried forward in more or less the same relative position during the entire period of growth. The nuclei of pollen tubes and latex vessels likewise follow the growth of the tip of the filament (**9, 19**). By centrifugation nuclei may be temporarily or, as in death, permanently displaced from their normal site; but, apart from this or other accident, the position of the nucleus is characteristic of the cell (**4**). The occurrence of nucleodesmata provides a possible explanation for this comparative stability.

Studies in the structure of the chloroplast and on plastids in general are at least as numerous as those on the nucleus (**14, 20, 22**). It is now evident that the current concept of the limiting surface of the chloroplast as an imperforate envelope stands in need of revision. Plastodesmata are similar to nucleodesmata in radial orientation, dimensions, and ubiquitous distribution. All fibrillae, however, appear to be of the same diameter; in other words, grouped or aggregated plastodesmata have so far not been observed. Like nucleodesmata in the nucleus, plastodesmata presumably aid in the traffic of materials between cytoplasm and plastid and may account for rapidity of transfer during active photosynthesis. The initial connection between cytoplasm and plastids is presumably established during plastid differentiation from chondriosomes or proplastids (**11, 12, 15**) and so far has not been observed.

Summary

1. In the giant nuclei of the endosperm and nucellus of *Echinocystis macrocarpa*, fibrillae, here named "nucleodesmata," interconnect nuclear and cytoplasmic networks across the nuclear membrane. Similar nucleodesmata occur in the nuclei of the epidermal cells of leaves, petioles, and stems of *Acanthus mollis*, *Tropaeolum nasturtium*, *Nicotiana tabacum*, and other species. It is therefore considered probable that nucleodesmata, like plasmodesmata, are ubiquitous.

2. In mature cells after division has ceased, the nuclear membrane is a finely perforate net, probably lipoproteic in nature and crystalline in submicroscopic structure.

3. Preceding ultimate disintegration and resorption, endosperm nuclei become lobed and may divide amitotically. During this increase in surface area, nucleodesmata remain ubiquitous on all lobed surfaces.

4. The relative stability of the position

of the nucleus within the living cell is explained, at least in part, by the presence of nucleodesmata.

5. It is considered possible that nucleodesmata may in reality be microcanals. Whatever their structure, they most probably function in the transport of materials across the nuclear membrane. They should, therefore, be significant in relation to the occurrence of the cytoplasmic nucleoproteins demonstrated by CASPERSSON and his colleagues and should also be discussed in connection with the differentiation and distribution of plasmagenes.

6. The limiting membranes of chloroplasts and other plastids are also finely perforate structures. "Plastodesmata," similar in structure to nucleodesmata, unite stroma and cytoplasm and presumably function actively in transport of material.

7. Since the visibility of the nucleus depends on partial dehydration, the nuclear volumes hitherto recorded give an entirely erroneous picture of the nucleus in action in the living cell.

8. Nucleoplasm and cytoplasm form a continuous network within the individual cell, an integral part of the protoplasmic reticulum of the plant as a whole.

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THE ROLE OF MECHANICS IN THE EVOLUTION OF THE HERBACEOUS PLANT STEM[1]

REYNOLDS B. SMITH

Introduction

An upright plant stem is a structure which, from time to time, undergoes bending stresses, and in which certain parts are continuously under stresses produced by the weight of the plant itself. Therefore, consideration of the adaptations which, in the course of evolution, have tended to increase a stem's mechanical efficiency should constitute a valuable addition to our knowledge of the development of herbaceous plants.

In many cases changes in structure which have increased physiological efficiency have also enhanced the mechanical efficiency of the stems of certain species. It has been the purpose in this investigation, however, to exclude the physiological considerations for the most part and to seek information concerning only the mechanical value of evolutionary adaptations.

Early studies of the development of the primary vascular bundles in stems and the study of consequent inception of secondary growth, both fascicular and interfascicular, led to the assumption that this must be the order followed in the evolutionary sequence. Such a hypothesis assumes that the herbaceous type is the more primitive and the woody type the more recent. Paleobotanical discoveries seem to indicate, however, that the reverse relationship holds true. HABERLANDT (5) suggested that the primitive woody cylinder was broken up into bundles by the thinning of the woody cylinder until the medullary and cortical tissues were brought into intimate contact at the leaf and branch gaps. Extensions upward and downward of the individual gaps would then account for the final breaking-up into separate vascular bundles. EAMES (2, 3) demonstrated that the mechanism of development of herbaceousness in members of the Rosaceae was by means of an increase in the number of aggregate rays found in the more primitive woody species of this family. JEFFREY (7) and JEFFREY and TORREY (8, 9) extended the studies of this type of development to include the Fagaceae and Betulaceae by indicating that, even in extremely woody stems, such a development was to be seen in the aggregation of the rays in the vicinity of outgoing leaf traces. Studies by WHITAKER (17) on the Compositae indicated that such a development from the woody to the herbaceous habit was a logical conclusion. JEFFREY (7) summed up the evidence for the aggregate ray theory. SINNOTT and BAILEY (13, 14) interposed certain objections to the theory of JEFFREY. They returned to the idea of HABERLANDT that the thinning of the xylem cylinder alone was responsible for herbaceousness.

The value of these adaptations in the survival of individual plant species has been assumed to be physiological. It is argued that increase in the volume of the ray tissue surrounding the leaf traces constitutes a more efficient storage system, since its position assures a minimum of translocation from centers of photosynthesis to points of storage. In considering this explanation, however, EAMES

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(3) raised the question: Why should extra storage be necessary in plants whose stems normally die after one season, as do stems of most herbaceous annuals and the aerial stems of most herbaceous perennials? Although physiological considerations undoubtedly enter into the value of these adaptations in species survival, it seems reasonable to believe that some further explanation of their roles can be found.

In reviewing the literature on the mechanical features of stems, one is impressed by the paucity of publications in this field. SCHWENDENER's *Das mechanischen Prinzip im Bau der Monocotylen* published in 1874 (12), is the principal work of this nature. SCHWENDENER's investigations were both mechanically and mathematically sound, and his measurements of tissue strengths produced results of the same order of magnitude as present-day tests on woody tissues.

The work of SCHWENDENER's students, particularly of WEINZIERL (16) and FIRTSCH (4), was confined almost entirely to measurements of the strength properties of various tissues. Nearly all succeeding work has drawn heavily upon the original development of the subject by SCHWENDENER and upon the refinements made by his students.

More recent information on the mechanics of plant stems has come as a by-product in studies in forest mensuration aimed at the development of tree form factors for use in estimating volumes of standing timber. Finally, very recent work by OPATOWSKI (11) has produced highly illuminating results in the application of theoretical mechanics to tree boles.

Material and methods

Eighty-two herbaceous and shrubby species, representing forty-one plant families, were selected for investigation (table 1). Cross sections and, in many cases, longitudinal sections of their stems were prepared for microscopic examination. Dehydration followed the method described by JOHANSEN (10), and the materials were imbedded in paraffin from tertiary butyl alcohol. In most instances the sectioning was done with a rotary microtome of standard type; some of the more woody stems were cut with a sliding microtome after imbedding in celloidin in the manner usually employed in anatomical work. In a few cases supposedly herbaceous stems proved to be so woody, or the xylem so hard, that sectioning in paraffin was difficult without further softening. To overcome this difficulty, the transverse surface of the stem was exposed by cutting across the paraffin block; the block was then immersed in 20% hydrofluoric acid for 24 hours, followed by a 24-hour washing in running water. Such treatment softened the sclerized tissues without causing any apparent damage to the softer tissues, which were probably protected by the more complete impregnation with paraffin. The sections were stained with safranin and Haidenhain's haematoxylin and mounted in Canada balsam.

Anatomical characteristics of stems

For the purposes of this investigation the general form of the stem and the quantitative distribution of tissues of a given kind in certain areas on the transverse surface are the important considerations; only occasionally are histological details of sufficient moment to be considered pertinent to the problem at hand. Thus the descriptions are mainly concerned with the arrangement of the tissues, and histological observations have been incorporated only when they are considered relevant. The descriptions are arranged in the order followed in table 1.

TABLE 1

SPECIES INVESTIGATED

Urticaceae
Humulus lupulus L.
Pilea pumila (L.) Gray
Aristolochiaceae
Asarum canadense L.
Aristolochia durior Hill.
Polygonaceae
Polygonum aviculare L.
Amaranthaceae
Amaranthus retroflexus L.
Phytolaccaceae
Phytolacca americana L.
Caryophyllaceae
Silene latifolia (Mill.) Britten & Rendle
Ranunculaceae
Anemone quinquefolia L.
Clematis virginiana L.
Actaea alba (L.) Mill.
Menispermaceae
Menispermum canadense L.
Berberidaceae
Podophyllum peltatum L.
Cruciferae
Brassica kaber (DC.) Wheeler
Rosaceae
Potentilla recta L.
Geum canadense Jacq.
Rubus odoratus L.
R. pubescens Raf.
Leguminosae
Medicago sativa L.
Desmodium bracteosum (Michx.) DC.
Lespedeza capitata Michx.
Linaceae
Linum usitatissimum L.
Euphorbiaceae
Ricinus communis L.
Euphorbia peplus L.
Celastraceae
Celastrus scandens L.
Balsaminaceae
Impatiens pallida Nutt.
Vitaceae
Parthenocissus quinquefolia (L.) Planch.
Malvaceae
Malva rotundifolia L.
Althaea rosea (L.) Gray
Hypericaceae
Hypericum perforatum L.
H. prolificum L.
Thymelaeaceae
Dirca palustris L.
Onagraceae
Oenothera biennis L.
Araliaceae
Aralia racemosa L.
Panax quinquefolium L.
Umbelliferae
Sanicula marilandica L.
Zizia aurea (L.) Koch
Taenidia integerrima (L.) Drude
Cornaceae
Cornus canadensis L.
Ericaceae
Kalmia polifolia Wangh.
Epigaea repens L.
Gaultheria procumbens L.
Chiogenes hispidula (L.) Torr. & Gray
Vaccinium oxycoccos L.
Apocynaceae
Vinca minor L.
Apocynum androsaemifolium L.
Asclepiadaceae
Asclepias tuberosa L.
A. syriaca L.
Convolvulaceae
Convolvulus sepium L.
C. sp.
Hydrophyllaceae
Hydrophyllum virginianum L.
Boraginaceae
Cynoglossum officinale L.
Myosotis scorpioides L.
Echium vulgare L.
Verbenaceae
Verbena urticaefolia L.
Labiatae
Agastache nepetoides (L.) Ktze.
Nepeta cataria L.
Physostegia virginiana (L.) Benth.
Monarda didyma L.
Mentha piperita L.
Collinsonia canadensis L.
Solanaceae
Solanum dulcamara L.
S. carolinense L.
Physalis virginiana Mill.
Scrophulariaceae
Verbascum thapsus L.
Veronica officinalis L.
Phrymaceae
Phryma leptostachya L.
Rubiaceae
Galium triflorum Michx.
Mitchella repens L.
Caprifoliaceae
Lonicera dioica L.
L. sempervirens L.
Linnaea borealis L. var. *americana* (Forbes) Rehd.
Dipsacaceae
Dipsacus sylvestris Huds.
Lobeliaceae
Lobelia siphilitica L.
L. kalmii L.

TABLE 1—*Continued*

Cucurbitaceae
Cucumis sativus L.
Compositae
Solidago gigantea Ait.
S. canadensis L.
Sericocarpus asteroides (L.) B. S. P.
Arctium minus Bernh.
Cichorium intybus L.
Prenanthes alba L.

Humulus lupulus.—This twining, herbaceous, perennial vine often attains a height of 25 feet. The xylem is cylindrical and incloses a narrow pith. The leaf traces are flanked by parenchyma in the form of wide rays passing radially through the secondary xylem. In the nodes the departing leaf trace is completely inclosed in parenchyma. The primary xylary bundles are flanked by bands of parenchyma which connect with 2-3-seriate rays in the secondary xylem.

Pilea pumila.—Clearweed is an erect annual with a succulent stem. The stem is rectangular with five vascular bundles evenly spaced across the narrow ends of the rectangle and a single large vascular bundle located on the center line just internal to the edge of each of the longer sides. The xylem is entirely primary, without secondary growth.

Asarum canadense.—The perennial portion of the stem is fleshy and creeping; the aerial portion is very short, with two long petioled leaves and a short peduncle bearing a single flower. The pith is scarcely distinguishable from the cortex. The xylem consists of bundles scattered in the ground tissue. A fascicular cambium produces a very small amount of secondary tissue; the vestigial interfascicular cambium is inactive.

Aristolochia durior.—Dutchmanspipe is a twining woody vine which seldom exceeds an inch in diameter but often attains a length of 30 feet. The xylem is cylindrical and incloses a narrow pith. It is broken into segments by large compound rays. The secondary xylem is characterized by large and numerous vessels.

Polygonum aviculare.—In this annual the stem is simple and seldom attains a length of more than 2 feet. The xylem cylinder incloses a pith, with a diameter of one and a half times its own thickness. The leaf traces and branch traces are inclosed by copious parenchyma in their outward passage.

Amaranthus retroflexus.—The stem is erect and sometimes reaches a height of 10 feet. The secondary xylem is irregularly broken by scattered tracts of parenchyma. In the upper internodes the xylem is dissected into irregular bundles by an even deeper invasion of parenchymatous tissue, and these bundles are accompanied by both internal and external bundles of phloem.

Phytolacca americana.—Pokeweed is a perennial herb, with a branching stem that attains a length of 4–12 feet. The secondary xylem forms a thin cylinder inclosing a massive pith. This xylem is divided into wide segments by multiple rays which pass through it radially external to each of the primary bundles. An active cambium is present.

Silene latifolia.—The stem of this perennial herb reaches a height of 6–18 inches; the thin xylem cylinder incloses a massive pith. The outgoing leaf traces are preceded radially through the secondary xylem by large multiple rays.

Anemone quinquefolia.—The stem is simple and erect and attains a height of 4–9 inches. The xylem is distributed in discrete bundles separated by wide tracts of parenchyma which appear to have been derived from sclerized tissue.

Clematis virginiana.—Virginsbower is

a vine which attains a length of 18 feet; it is fluted in cross section in the younger portions; in the older parts the cross-sectional outline becomes hexagonal. The xylem consists of twelve discrete bundles separated by wide medullary rays. The cells of these rays differ from the medullary parenchyma in their radial elongation. The vessels are conspicuous for their large diameters.

Actaea alba.—This erect perennial herb has a stem that attains a length of approximately 2 feet. The xylem consists of a ring of bundles separated by wide parenchymatous rays. The ray cells are somewhat smaller in diameter than those of the pith. Secondary thickening occurs in relatively small amounts.

Menispermum canadense.—This slender, somewhat woody vine occasionally attains a length of 15 feet. The xylem cylinder is broken into discrete bundles separated by 1–3-seriate medullary rays; the cells of the ray parenchyma are elongated radially. The vessels are conspicuous for their large size.

Podophyllum peltatum.—This plant has an erect stem, 1–1½ feet in length, which develops from a rootstock. The leaves are peltate. The vascular bundles are scattered throughout the parenchymatous ground tissue. A fascicular cambium produces small amounts of secondary thickening, but there is no trace of an interfascicular cambium. The arrangement of the bundles is suggestive of the conditions found in some monocotyledonous stems.

Brassica kaber.—This annual has an erect stem 1–2 feet tall. The xylem cylinder has a cross-sectional thickness of only one-third that of the diameter of the pith. The primary bundles are flanked on either side by parenchyma identical with that of the medullary tissue. These radial tracts of ground tissue, however, are not continued into the secondary xylem by development of aggregate or multiple rays. An extensive secondary thickening is evident.

Potentilla recta.—Cinquefoil has an erect stem which attains a height of 1–2 feet. The xylem cylinder is moderately thick, relative to the diameter of the pith. Some aggregate rays are present radially external to the leaf trace bundles.

Geum canadense.—The stout erect stem attains a length of 2½ feet. The vascular tissue consists of discrete bundles separated by wide tracts of parenchyma identical with the pith. The xylem elements in the leaf trace bundles are in definite radial alignment. This species appears to represent an extreme in herbaceousness in the Rosaceae.

Rubus odoratus.—The stem of thimbleberry is erect and shrubby and often attains a height of 5 feet. The xylem cylinder, which incloses a massive pith, is relatively thin and is broken into radial tracts by large aggregate rays. In the vicinity of the leaf traces the externally adjoining secondary xylem displays fewer vessels and a greater concentration of rays and fibers.

Rubus pubescens.—This plant is herbaceous, with a trailing, or ascending, armed stem 6–18 inches in length. The xylem cylinder is thin, but the pith is not relatively massive. Aggregate rays in the secondary xylem are confined for the most part to the regions externally adjoining the leaf traces. This species is more herbaceous, and the stem more strongly angled (nearly square), than in *R. odoratus*.

Medicago sativa.—The stem of alfalfa is ascending or decumbent and may attain a height of 1½ feet, occasionally taller. The upper stem is square, the lower pentagonal, in transverse section. The

xylem consists of discrete bundles of vascular tissue consisting of vessels, fibers, and rays. The intervening tracts of secondary xylem are almost entirely fibrous, with very few rays and no vessels.

Desmodium bracteosum.—This herbaceous perennial has a stem 2–6 feet in length. The xylem cylinder is relatively thin and incloses a massive pith. Large aggregate rays are present in the secondary xylem external to the leaf traces.

Lespedeza capitata.—The erect, wandlike stem often attains a length of 6 feet. The xylem cylinder is massive, and in the specimen observed there were zonations suggestive of annual rings. No aggregation of rays contiguous to the outgoing leaf traces was evident. Cambial activity, however, appears to be inhibited external to the leaf traces, with a consequent thinning of the xylem in these regions until gaps are formed through which the traces pass outward into the petioles.

Linum usitatissimum.—Flax is an erect, branching annual with a terete stem approximately 20 inches in length. The xylem is thin relative to the diameter of the pith. The rays are mostly uniseriate, but the number per unit area increases in areas external to outgoing leaf traces. These areas are in turn separated from adjoining xylem by 3–4-seriate multiple rays.

Ricinus communis.—The tall stout stem is at first herbaceous but later becomes woody; it attains a length up to 15 feet. The diffuse porous xylem is a stout cylinder, with relatively sparse vessels. The rays are 1–2-seriate, and the xylem is largely fibrous in nature. No tendency toward compounding or aggregation of the rays was observed.

Euphorbia peplus.—The erect succulent stem varies from 6 inches to 3 feet in length. The xylem consists of a very thin cylinder which incloses a massive pith. The secondary xylem is almost completely fibrous, with very few vessels; the rays are invariably uniseriate. *E. peplus* exhibits every appearance of herbaceousness achieved by progressive reduction in the amount of secondary growth without any proliferation of the ray tissue.

Celastrus scandens.—This twining woody vine often grows to a height of 25 feet, but, where support is lacking, it may assume a prostrate habit. The xylem cylinder is relatively thick; the hollow pith is large. The xylem is broken by multiseriate rays arranged alternately with the primary xylem bundles. There is a slight aggregation of the rays in regions just external to the outgoing leaf traces.

Impatiens pallida.—The stem of this annual is stout and succulent; it reaches a length of 2–5 feet. The secondary xylem cylinder is extremely thin relative to the pith. The xylem is mostly fibrous except in the immediate proximity of the primary bundles. *I. pallida* is very similar to *Euphorbia peplus* in its histological topography; it appears to have become herbaceous through reduction in the amount of secondary thickening.

Parthenocissus quinquefolia.—This high-climbing or trailing vine often attains a length of 30 feet. The secondary xylem is cylindrical and incloses a relatively small pith. It is divided into broad segments by multiseriate rays. The vessels are numerous, uniformly distributed, and large in proportion to the diameter of the stem.

Malva rotundifolia.—This annual or biennial has a branched stem which reaches a length of 4–12 inches. The cylinder of xylem is thin relative to the massive pith. The xylem is separated by wide multiseriate rays into radial tracts of varying width. The broad rays are

particularly wide in the flanks of the secondary xylem radially external to the outgoing leaf traces.

Althaea rosea.—Except as to its larger size, the stem is identical in topography to that of *M. rotundifolia* just described.

Hypericum perforatum.—This plant is herbaceous from a perennial woody base; its stem attains a length of 1–2 feet. It is in every respect but size similar in anatomy to *H. prolificum.*

Hypericum prolificum.—Shrubby St. Johnswort is woody in texture and diffusely branched. It attains a length of 1–3 feet. The pith is relatively narrow in proportion to the thickness of the cylinder of diffuse porous xylem inclosing it. The leaf traces are abutted externally by secondary xylem containing broad rays. In the vicinity of the departure of the leaf traces the xylary rays of the adjacent secondary xylem curve inward toward the opening leaf gaps.

Dirca palustris.—Leatherwood is a shrub growing to a height of 6 feet. The stem is typically woody throughout with a small pith. Included phloem occurs as scattered, irregular patches in the xylem. No tendency toward herbaceousness is registered.

Oenothera biennis.—The erect, stout stem is sometimes branched and attains a length of 1–6 feet. The pith is relatively narrow, and the xylem forms an inclosing cylinder of a highly fibrous nature. There appears to be no proliferation of the ray tissue in the vicinity of the leaf traces; instead, the xylem cylinder undergoes thinning external to the departing leaf traces.

Aralia racemosa.—The erect, herbaceous stem is 1–6 feet in length. Its vascular tissue is arranged in three series of discrete bundles forming three concentric circles. The development of the primary xylem of the inner two series is exarch, while that of the outer series is endarch. The inner series of bundles are imbedded in sclerenchyma.

Panax quinquefolium.—Ginseng is an erect, perennial herb with a stem 8–15 inches in length. In the lower stem there are two concentric rings of discrete vascular bundles. The bundles of the inner series consist mostly of protoxylem and phloem; the phloem is variously oriented with respect to the xylem. The vascular bundles of the outer series are of the type, as to both development and orientation, usually found in herbaceous dicotyledons.

Sanicula marilandica.—This herbaceous perennial has a stout stem often attaining a length of 4 feet. The vascular tissue is arranged in a ring of discrete bundles. The bundles are unique in having a sclerenchymatous sheath inclosing the xylem and phloem and also separating the two.

Zizia aurea.—The erect stem is 1–1½ feet in length. In the lowest portion the xylem forms a cylinder inclosing the pith; in the upper portions the vascular tissue is broken into bundles by wide radial tracts of parenchyma. The upper stem is octagonal in transverse section. The outgoing leaf traces are sheathed by parenchyma except distally where small bundles of sclerenchyma occur. Schizogenous oil ducts are numerous throughout the stem cross section.

Taenidia integerrima.—This perennial herb has an erect stem which attains a length of 1–3 feet. The stem is round near its base but ten-angled in its upper portions. The vascular tissue occurs in discrete bundles, and the intervening tissue in the secondary growth is sclerenchymatous. Small schizogenous oil ducts are present internal to the vascular bundles. Tiny strands of phloem surrounded by a narrow sheath of sclerenchyma some-

times accompany these ducts. Where such oil ducts occur in the highest portions of the stem, they have entirely lost the investing phloem and sclerenchyma.

Cornus canadensis.—The stem is woody only at the base and attains a length of 3–9 inches. In shape it suggests an I-beam, with a rectangular vascular system in the center of the web. The xylem is continuous and relatively thin, sparsely diffuse-porous, and relatively fibrous. The uniseriate rays are regularly and uniformly distributed. In the upper portions the stem becomes square in transverse section while the xylem remains rectangular.

Kalmia polifolia.—This woody shrub is 6 inches to 2 feet tall. It is entirely woody and shows no tendency toward herbaceousness except for a slight compounding of the rays just external to departing leaf traces.

Epigaea repens.—This prostrate or trailing woody shrub may attain a length of several feet. The xylem is cylindrical and continuous with a relatively large pith in its upper portions. In the region of outgoing leaf traces the xylem becomes very eccentric in cross section, and there appears to be a tendency toward concentration of the rays in the xylem adjacent to the outgoing traces.

Gaultheria procumbens.—Wintergreen is a woody perennial with creeping or subterranean stems which produce erect branches bearing leaves, flowers, and fruits. The xylem is cylindrical and slightly thinner than the diameter of the pith. There is a thinning of the xylem and an increase in the diameter of the pith at the nodes. The xylary rays are mostly uniseriate except in the vicinity external to the departing leaf traces, where they may be 2–3-seriate. The departing leaf traces are accompanied by wide tracts of parenchyma which encircle them. These leaf gaps are comparatively short, however, and at successive levels upward seem to result from thinning of the xylem cylinder until pith and cortex are virtually in contact. This eventually breaks the xylem into bundles in the higher nodes.

Chiogenes hispidula.—This prostrate, evergreen shrub has a creeping, branched stem. The xylem, which is narrower in cross section than the diameter of the pith, is diffuse-porous, with uniseriate rays. The rays exhibit a tendency toward compounding in the vicinity of the leaf traces. As the departing leaf traces approach the cortex, the secondary xylem becomes thinner until it finally opens into a leaf gap.

Vaccinium oxycoccos.—This trailing shrub has a very slender, creeping stem which produces erect branches bearing leaves, flowers, and fruits. The xylem cylinder is only half as thick as the diameter of the pith. The xylem is diffuse-porous, with numerous uniseriate rays which show a tendency toward compounding in the regions external to departing leaf traces.

Vinca minor.—Periwinkle is a perennial, often evergreen, plant with trailing stems. The xylem is square in cross section and incloses a pith whose diameter is four times the thickness of the xylem layer. The diffuse-porous xylem has relatively few vessels and 1-, 2- (occasionally 3-) seriate rays. Secondary growth seems to be inhibited slightly in the xylem regions external to departing leaf traces. In the nodal region the stem is elliptical, with the major axis of the ellipse directed toward the oppositely situated leaf traces. The leaf gap is comparatively short vertically and appears to result from thinning of the xylem cylinder until pith and cortex are virtually in contact.

Apocynum androsaemifolium.—This perennial, branching herb has an annual stem 1–4 feet in length arising from a horizontal rootstock. The xylem cylinder is narrow relative to the thickness of the large pith, with a ratio of about four to one. The xylem is diffuse-porous with uniseriate (occasionally biseriate) rays. The upper stem is elliptical in cross section with the departing leaf traces at either end of the major axis. As in *V. minor*, the left gaps appear to result from thinning of the secondary xylem.

Asclepias tuberosa.—Butterfly weed is a perennial herb with a stout, erect stem, 1–2 feet in length. The xylem consists of discrete collateral bundles of primary wood, with scarcely any evidence of secondary thickening. These are separated by tracts of parenchyma identical to that of the pith. The cambium, if present, appears to be vestigial.

Asclepias syriaca.—Milkweed is a herbaceous perennial with a stout, erect stem, 3–5 feet long. In the lower portions the xylem is cylindrical and incloses a pith with a diameter about five times the thickness of the cylinder. Considerable secondary thickening is present. The xylem rays are uniseriate, occasionally biseriate, and rarely triseriate. The secondary xylem in the vicinity of the leaf traces displays a larger number of rays than equal areas of the xylem elsewhere. In the region of the node, as the outgoing leaf trace approaches the cortex, much of the adjacent xylem is distorted into swirls of fibers mixed with parenchyma, resembling in some respects typical aggregate rays on an enlarged scale.

Convolvulus sepium.—This herbaceous perennial has a climbing or occasionally trailing stem, 3–10 feet in length. The xylem cylinder incloses a pith with a diameter nearly ten times its own thickness. The lower parts of the stem tend toward a triangular cross section. Extensive tracts of secondary xylem appear at nearly opposite portions of the stem. In its upper portion the stem is more definitely triangular in cross section, with copious secondary thickening in the angles. The xylem is diffuse-porous with uniseriate rays. In the regions of departing leaf traces the secondary thickening appears to be so inhibited that the pith and cortex are virtually in contact, resulting in the formation of leaf gaps.

Convolvulus sp.—This specimen was not identified as to species except that it was definitely not *C. sepium*. The stem contained much more secondary thickening than that of the previously described species but was otherwise similar.

Hydrophyllum virginianum.—This perennial herb has an aerial stem which attains a length of 1–3 feet. The stem is more or less elliptical in shape except for two auricular outgrowths at one side which is somewhat flattened. The vascular system, which consists of discrete bundles, is located near the center of a relatively massive parenchymatous ground tissue. Only a few cells of secondary xylem are produced by a weakly active cambium.

Cynoglossum officinale.—Houndstongue is a biennial herb with a stout, erect stem, $1\frac{1}{2}$–3 feet in length. The diffuse-porous xylem, which is devoid of rays, forms a cylinder inclosing a pith with a diameter ten to twelve times its thickness. The leaf gaps are produced by a gradual thinning of the xylem adjacent externally to the leaf traces. In these areas the xylem is devoid of vessels and appears to consist solely of tracheids and fibers.

Occasionally, outgoing leaf traces appear to arise as amphicribral bundles internal to the xylem cylinder. These depart through the xylem cylinder in a

manner identical with that of the normal bundles.

Myosotis scorpioides.—This perennial herb has a decumbent or ascending aerial stem 6–18 inches in length. The xylem cylinder is very narrow, inclosing a pith with a diameter twenty-five times its thickness. Rays are wanting in the secondary xylem. Activity of the cambium is apparently inhibited in the areas external to the leaf trace bundles which results in thinning of the xylem cylinder. Vessels are lacking in these regions; elsewhere the xylem is diffuse-porous.

Echium vulgare.—This bristly-hairy biennial has a stout, erect stem, 1–2½ feet in length. The xylem cylinder surrounds a pith with a diameter over four times its thickness. The xylem is diffuse-porous and devoid of rays. The xylem external to the outgoing leaf traces lacks rays, and the leaf gaps appear to be formed by inhibition of the secondary thickening. At higher levels the stem becomes definitely hexagonal in outline.

Verbena urticaefolia.—The stem of this erect, branched perennial is approximately square in cross section with round corners. The xylem has the same topography, and its thickness is only about one-tenth of the cross-sectional dimensions of the pith. The xylem in the corner regions is diffuse-porous; in these areas the rays are uniseriate and occasionally biseriate. Along the flattened sides in the vicinity of the outgoing leaf traces both rays and vessels are wanting. Leaf gaps appear to be formed by a thinning of the secondary xylem through inhibition of cambial activity external to the departing leaf traces. The corners of the stem are reinforced by copious collenchyma tracts in the cortex.

Agastache nepetoides.—This herbaceous perennial is one of the tallest of the native mints; the nearly square stem often attains a height of 5 feet. The cross-sectional dimensions of the pith vary from twenty to nearly a hundred times the thickness of the inclosing square of xylem. At the corners the xylem is diffuse-porous with uniseriate rays. Along the flattened faces the xylem is devoid of rays throughout and of vessels except immediately external to the departing leaf traces. Each of the flangelike projections at the corners is reinforced by a large distal tract of collenchyma.

Nepeta cataria.—Catnip is a herbaceous perennial with a square, erect stem, 2–3 feet in length. The xylem, which is also square in general outline, incloses a pith with dimensions six to forty times that of the thickness of the xylem. Xylary rays are wanting throughout. The xylem is diffuse-porous in the corners, but vessels are wanting in the portions underlying the flattened sides of the stem except distally to the leaf traces.

Near the uppermost nodes the cambial activity appears to be more suppressed, and the leaf gaps seem to continue a greater distance above each node than at lower levels. The large bundles at the corners are consequently more nearly discrete, and the stem is hence more herbaceous in nature.

Physostegia virginiana.—This perennial herb has a stem which is erect, rather stout, and attains a length of 1–4 feet. As in most members of the mint family, the stem is square in cross section. The xylem forms a relatively thin layer about a massive pith of dimensions 4–8 times greater than the thickness of the xylem. The xylem is diffuse-porous in the corners, but vessels are absent under the flattened sides except distally to the leaf traces. The xylem is devoid of rays throughout the cross section. The cambium is continuous, but its activity is inhibited along the flattened sides in the

vicinity of the leaf traces. In the region of the uppermost nodes the gaps appear to continue a relatively greater distance above the node than at lower levels; hence, the stem becomes more herbaceous at these levels.

Monarda didyma.—Oswegotea is an aromatic, perennial herb with a square, stout, ascending stem 2–3 feet in length. The pith of this species is five to ten times greater in cross-sectional dimensions than the xylem. The xylem at the corners is diffuse-porous and has uniseriate or, occasionally, biseriate rays. The xylem internal to the flattened faces is devoid of rays and possesses vessels only in the immediate region of the leaf traces. The cambial activity throughout the stem appears to be limited.

Mentha piperita.—Peppermint is a herbaceous perennial with an erect, branched stem which is square in cross section and often attains a length of 3 feet. The xylem varies in thickness from one-tenth to one-thirtieth the cross-sectional dimensions of the pith. The xylem of the corners is diffuse-porous and has uniseriate rays. Rays and vessels are lacking in the secondary xylem internal to the flattened faces except just distal to the leaf traces where a few vessels and uniseriate rays may occur. The stem becomes more nearly round at the nodes, but there is no reappearance of vessels or rays in regions corresponding to those in internodes where these structures are lacking.

Collinsonia canadensis.—The stem in this perennial herb is stout, branched, erect, and 2–5 feet in length. It is square in cross section but with much more rounded corners than the stems of the mints previously described. The thickness of the xylem is from one-seventh to one-twentieth the cross-sectional dimensions of the pith. Vessels are restricted to the corners and to the regions along the flattened faces just distal to the leaf trace bundles. The uniseriate rays are confined to the corners except for an occasional ray in the xylem external to a leaf trace.

Solanum dulcamara.—This perennial liana has a climbing or scrambling stem 2–8 feet in length. The pith has a diameter approximately the same as the thickness of the xylem cylinder. The first annual ring of xylem is diffuse-porous, with the pores somewhat more numerous in areas just external to the leaf trace bundles. In succeeding annual rings the number of vessels is noticeably reduced in the areas between the leaf trace regions. The leaf gaps are comparatively short longitudinally and appear to be formed by thinning of the secondary xylem until pith and cortex are virtually in contact.

Solanum carolinense.—This perennial species has an erect, branched stem 1–4 feet in length, which is quite succulent at first but eventually becomes woody. It is somewhat oval in cross section. The thin xylem cylinder flanks the massive pith and becomes much thicker at the ends, where it adjoins the outgoing leaf traces. The xylem along the flanks of the oval is diffuse-porous and possesses numerous uniseriate rays. In the vicinity of the leaf traces it is almost entirely fibrous except for vessels just external to the outgoing bundles. Internal phloem in the form of small bundles is present throughout the periphery of the pith; these bundles are usually accompanied by small masses of sclerenchyma on the inner side. The wide leaf gaps are apparently formed by thinning of the secondary xylem cylinder. These continue upward to the next higher node, where a thin layer of secondary xylem again appears which increases at higher levels. At first this attenuated layer is largely fibrous, but rays and vessels reappear farther up the stem.

Physalis virginiana.—Although this plant is perennial, its aerial stem is fleshy, erect, and dichotomously branched, and attains a length of 1½–3 feet. The stem is rounded in cross section but displays a tendency to become triangular. It is flanged with four winglike outgrowths near the base; these become rudimentary at higher levels. The xylem cylinder has a thickness one-eighth to one-third the diameter of the pith. The xylem is diffuse-porous in the areas external to the leaf traces; there are few vessels in the intervening areas. The rays are uniseriate or occasionally biseriate and are sparse in the regions between leaf traces. Secondary thickening in the region of the traces appears to be suppressed. Small bundles of internal phloem are closely associated with the protoxylem. In the upper stem the cross section becomes more truly triangular.

Verbascum thapsus.—This biennial herb has an erect, stout stem which reaches a length of 2–7 feet. The xylem cylinder has a thickness of one-third to one-fifth the diameter of the pith. The xylem is diffuse-porous, with the pores more numerous toward the outer portions of the ring. In the region of the leaf traces the number of vessels in the secondary xylem is reduced except just external to them, where there is also an increase in the number of rays. At higher levels the vessels of the xylem are mostly confined to regions distal to the leaf traces and here the rays in the xylem regions between leaf traces appear to arise in the outer quarter of the xylem ring. Small bundles of internal phloem are closely associated with the protoxylem.

There is a tendency toward a square outline in the lower stem, which changes to triangular in the upper levels.

Veronica officinalis.—This perennial herb has a simple stem which attains a length of 3–10 inches. The diffuse-porous xylem cylinder is thin relative to the massive pith. The uniseriate rays as well as the vessels are uniformly distributed. The secondary xylem undergoes no significant modifications in the vicinity of the leaf traces, except for a slight decrease in the number of vessels in these regions. The gap is formed by a marked thinning of the xylem cylinder until pith and cortex appear to come into contact.

Phryma leptostachya.—This perennial herb has an erect, four-angled stem, 1½–3 feet tall. The xylem is arranged almost exactly as in the labiate and verbenaceous species already described. A single leaf trace occurs midway of each of the flattened sides; immediately external to these traces the xylem is nearly devoid of pores, but elsewhere it is diffuse-porous. Rays are also scarce just external to the leaf traces.

The assignment by HUTCHINSON (6) of this monotypic genus to the Verbenaceae on a floral basis seems justified as well by the comparative anatomy of the stem.

Galium triflorum.—This perennial herb has an erect or partially procumbent stem which may reach a length of 3 feet. In cross section the stem is square with a flangelike wing in each corner which is composed of collenchyma. The xylem cylinder is relatively thin and incloses a thick pith. The rays are uniseriate or biseriate. They increase slightly in number just external to the leaf traces.

Mitchella repens.—Partridgeberry is a creeping herb with a slender stem, 6–12 inches in length, which is compressed. The leaves are attached in the plane of compression. The xylem is elliptical, and the leaf traces depart from the ends of the major axis. The xylem is diffuse-porous except just external to the leaf traces, where vessels are wanting. The uniseriate

and biseriate rays are slightly more numerous in these same regions.

Lonicera dioica.—This species is twining or shrubby, with a terete stem 3–10 feet in length. The xylem cylinder surrounds a relatively large pith, and the xylem is diffuse-porous, with large vessels. In the regions external to the leaf traces the xylem is devoid of vessels, and in these areas the rays increase in number; most of the rays are 3–4-seriate.

Lonicera sempervirens.—This climbing vine or shrub may attain a height of 10–15 feet. The xylem is diffuse-porous with large vessels. There is a tendency for the rays to become grouped in radial tracts flanking the areas just external to the leaf traces. In these areas there is also a slight increase in the volume of ray tissue, but this increase is less in amount than in homologous areas of the stem of *L. dioica*.

Linnaea borealis var. *americana*.—Twinflower is a slender, creeping, woody herb with a stem 6 inches to 3 feet in length. The relatively narrow xylem cylinder incloses a large pith. The xylem is diffuse-porous, with large vessels. In the regions external to leaf traces vessels are wanting, but the larger rays increase in number and in width in these same regions.

Dipsacus sylvestris.—Teasel is a biennial herb with a stout, prickly stem, 3–6 feet in length, which is hexagonal in cross section. The xylem is relatively thin and incloses a massive pith. The uniseriate rays are evenly distributed except in the regions external to the leaf traces where the xylem is entirely fibrous. In areas between the fibrous tissue the xylem is diffuse-porous.

Lobelia siphilitica.—This species is a perennial herb; its stem is stout and usually simple and attains a length of 1–3 feet. The relatively thin xylem cylinder incloses a massive pith. The xylem is diffuse-porous except adjacent to the leaf traces, where it is entirely fibrous. Rays are extremely scarce. The leaf gaps are formed by transformation to parenchyma of the fibrous tissue just external to the leaf traces.

Lobelia kalmii.—In habit this species is very similar to *L. siphilitica* except that the stem rarely attains a length of more than 20 inches. The xylem cylinder is narrow relative to the massive pith. The secondary xylem is almost entirely fibrous, with only a few scattered vessels. Ray parenchyma is scarce and confined to the stem angles. Leaf gaps are formed by the transformation of the xylem just external to the leaf traces into parenchyma.

Cucumis sativus.—The common cucumber is an annual plant with a rough, trailing stem which may reach a length of 6–15 feet. The stem is shallowly five-lobed and, hence, nearly pentagonal in cross section. The vascular tissue is in ten discrete bundles separated by wide tracts of parenchymatous ground tissue. No interfascicular cambium is evident, and the fascicular cambium is apparently vestigial, with secondary growth confined to a few cells.

This species appears to have achieved a herbaceous condition almost coequal with that of many monocotyledons.

Solidago gigantea.—This perennial herb has a stout, erect stem 3–8 feet in length. The xylem appears to be annular but consists of discrete vascular bundles separated by tracts of fibrous secondary tissue. There is a tendency toward a hexagonal cross-sectional outline in the stem. The first-formed secondary xylem in the fascicular regions is diffuse-porous; the homologous interfascicular xylem is entirely fibrous. The secondary xylem formed subsequently, both fascicular

and interfascicular, is entirely fibrous. Schizogenous oil ducts and isolated sclerenchymatous fibers occur in the phloem.

Solidago canadensis.—This species is similar in habit to the preceding species except that the stem is usually shorter. Its anatomy is also similar to that of *S. gigantea* except that some pores occur in the secondary xylem in the interfascicular regions.

Sericocarpus asteroides.—This perennial herb has an octagonal stem. The vascular tissue is distributed in discrete bundles separated by tracts of thin-walled fibrous tissue. Cambial activity is weak, and hence the secondary tissue is small in amount. Schizogenous oil ducts are present in pith and cortex as well as in the fibrous tracts between the bundles. The epithelial tissue of these ducts is suggestive of phloem.

Arctium minus.—Burdock is a biennial herb with a somewhat octagonal stem, 2–5 feet in length. The vascular tissue is arranged in two series of eight bundles each; the eight larger bundles occupy the stem angles, while eight smaller ones are inserted in the cortex between the first eight. The large pith is entirely hollow. The xylem of the bundles is diffuse-porous. There is no apparent interfascicular cambium, and the fascicular cambium produces little secondary thickening. No oil ducts were found.

Cichorium intybus.—This perennial herb has a stiff stem 1–3 feet in length. The stem is usually hexagonal in cross section but occasionally five- or seven-angled. The xylem appears annular but is actually composed of bundles of vascular tissue separated by tracts of fibrous tissue. A strongly active cambium occurs in both fascicular and interfascicular regions. Schizogenous oil ducts occur regularly in the cortex and occasionally in the phloem.

Prenanthes alba.—This perennial herb has an upright stem 2–5 feet in length. As in the preceding species the xylem appears annular but is actually composed of discrete vascular bundles separated by tracts of fibrous tissue. The secondary thickening in the interfascicular regions is sparse compared with that produced in the fascicular portions. No oil ducts are present.

Mechanical principles involved in stem structures

In discussing the structural evolution of plants, it is necessary to define the mechanical principles involved, for they may have a bearing on plant structures. Such definitions are most easily and concisely expressed in mathematical terms. Although the mathematical development does not necessarily follow the biological sequence, nevertheless, it provides the most logical means of approaching and describing the evolutionary structural development.

Beams

Circular beams.—A plant stem, under the action of wind or other bending forces, the line of action of which is at right angles to the long axis, is a cantilever beam and can be analyzed as such. The basic equation for such a beam is

$$S = \frac{Mu}{I}, \qquad (1)$$

where S is the stress per unit area at u distance from the neutral axis, M is the bending moment, and I is the moment of inertia of the cross section.

Insertion of the proper moments of inertia in this equation gives expressions for the stress on any element at u distance from the neutral axis for beams of

any cross-sectional outline. For a solid cylindrical beam the equation becomes

$$S = \frac{4Mu}{\pi r^4}, \qquad (2)$$

where r is the radius of the beam. For a hollow cylindrical beam the equation becomes

$$S = \frac{4Mu}{\pi (r_1^4 - r_2^4)}, \qquad (3)$$

where r_1 and r_2 are, respectively, the outer and inner radii of the beam.

These equations show that the stress at any point is directly proportional to the external moment of force M and the distance u of that point from the neutral axis and is inversely proportional to the moment of inertia of the cross section about the same axis. Hence, the most effective arrangement of the strengthening material in any beam is that combining the greatest moment of inertia with the most effective distribution of material. This condition is fulfilled when the center of mass of the strengthening material is at its greatest distance from the neutral axis.

For purposes of comparison of different types of beams a combination of the cross-sectional moment of inertia I and the farthest element from the neutral axis at a distance c provides a convenient factor. This is called the section modulus Z. Its value, expressed as an equation, is

$$Z = \frac{I}{c}. \qquad (4)$$

In this equation c is simply a special case of the value u in the more general equation.

The above equation supplies a useful means of comparing a solid cylindrical beam with a hollow cylindrical beam of equal cross-sectional area of strengthening material.

By substitution of the values for I from equations (2) and (3), it is evident that the section modulus for a solid beam is

$$Z = \frac{\pi r^4}{4r},$$

and for a hollow beam

$$Z = \frac{\pi (r_1^4 - r_2^4)}{4 r_1}.$$

Since the condition has already been stated that the cross-sectional areas are equal,

$$\pi r^2 = \pi (r_1^2 - r_2^2),$$

and both of these quantities can be replaced by the cross-sectional area A. The two equations can then be written

$$Z = \frac{A r}{4}$$

and

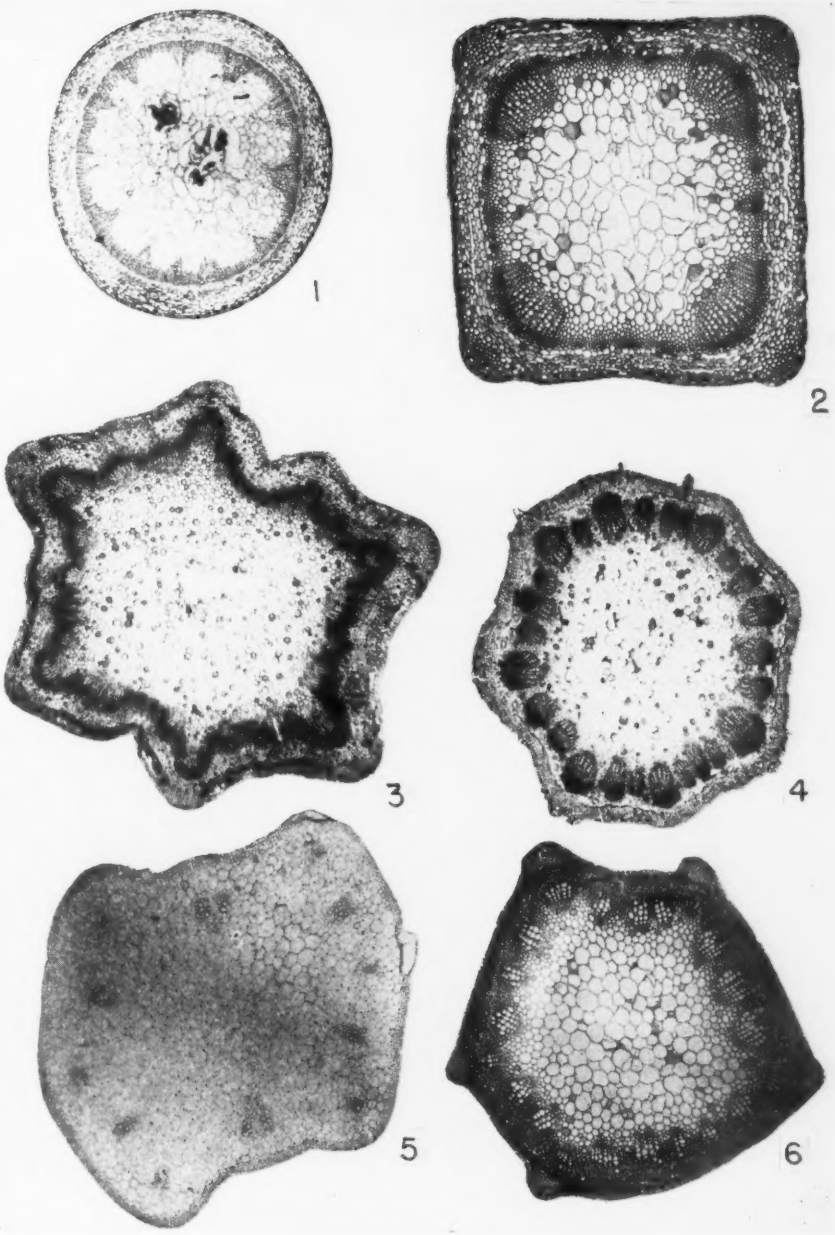
$$Z = A \frac{(r_1 + r_2^2 / r_1)}{4}.$$

By the original conditions also, r_1 must be larger than r, otherwise r_2 must be zero, and the hollow beam will be identical with the solid beam. Also, the fraction

$$\frac{r_2^2}{r_1}$$

must be positive, otherwise the beam does not exist. From this it follows that the section modulus for the hollow beam is always greater than that of a solid beam of equal cross-sectional area, and it is therefore more efficient.

Examples of contrasting sections of nearly equal cross-sectional areas of xylem are found in stems such as *Euphorbia peplus* (fig. 1) and very small members of the Ericaceae which remain small in diameter but extremely woody



FIGS. 1–6.—Cross sections of stems. Fig. 1, *Euphorbia peplus*, upper internode; fig. 2, *Mentha piperita*, upper internode; fig. 3, *Dipsacus sylvestris*, upper internode; fig. 4, *Sericocarpus asteroides*, lower internode; fig. 5, *Pilea pumila*, lower internode; fig. 6, *Medicago sativa*, upper internode.

in character with a xylem cylinder which is practically homogeneous.

BEAMS OF POLYGONAL CROSS SECTION.—A comparison of beams of square, hexagonal, and octagonal cross section with those of circular cross section reveals that the polygonal types are in many cases the more efficient in the application of available strengthening materials. Comparison of these types is simplified by again resorting to section moduli. Substitution of the left-hand member of equation (4) into equation (1) results in the expression

$$S = \frac{M}{Z}.$$

An analysis of this equation indicates that for any given moment M the resisting stress must vary inversely as Z, and, hence, of two beams having the same volume, the one having the larger section modulus is the more efficient.

The section modulus for a square with the neutral axis passing through the center and perpendicular to two sides is

$$Z = \frac{s^3}{6},$$

where s is the length of a side. The same factor for a circular section is

$$Z = \frac{\pi r^3}{4},$$

where r is the radius of the section. By substitution, in terms of the cross-sectional area A, these equations become, respectively,

$$Z = \frac{A s}{6}$$

and

$$Z = \frac{A r}{4}.$$

For comparison these equations must be expressed in similar terms. The relation between the side s of a square and the radius r of a circle of equal area is

$$s = \sqrt{\pi}\, r.$$

Substituting this value in the section modulus equation for the square results in

$$Z = \frac{\sqrt{\pi}\, A\, r}{6}.$$

Upon evaluation of the two similar equations, we find for the circle

$$Z = \frac{A r}{4} = 0.250\, A\, r$$

and for the square

$$Z = \frac{A r}{6} = 0.295\, A\, r.$$

From these two values it becomes evident that the square beam under the conditions stated is 1.18 times as efficient as the beam of circular cross section. The section modulus for a square with the neutral plane through the center and two opposite vertices can be shown by the same method to be only 0.84 times as efficient as the circular type. It is thus advantageous for such square stems as those of the mints (fig. 2) to have the mass of strengthening material in the corners, to oppose any bending stresses about a neutral plane passing through opposite corners.

Table 2 shows the computed sectional moduli for four types of cross sections in terms of a circle of equal area as well as the ratio of efficiency relative to such a circle.

Certain herbaceous plants with exceedingly small amounts of woody tissue have triangular stems, but here, because of the lack of symmetry about any given axis, the section modulus is low in efficiency as compared with that of a circular stem of equal cross-sectional area.

Removal of material to a depth of 0.011 of the diameter on opposite sides of a circular cross section, thus forming a rectangle with curved ends, results in an increase of 0.7% in the section modulus (15); certain plant stems (*Mitchella repens*, for example) possess this type of section.

BEAMS OF CONSTANT STRENGTH.—SCHWENDENER (12) showed that the longitudinal outline of a monocotyledonous stem approximates the shape of a beam, the radius of which at any point can be expressed by the equation

$$r = \sqrt[3]{\frac{x}{l}},$$

where r is the radius at any point, x is the distance of the point from the apex of the stem, and l is the total stem length. As expressed, this equation is satisfied only if the radius of the base is assigned a value of unity. To make the equation general, the basal radius must be shown in the equation as a constant of proportionality, thus:

$$r = R\sqrt[3]{\frac{x}{l}}.$$

This equation is that for the outline of a beam the section modulus of which varies proportionately with the bending moment at the corresponding point within its length. This equation also provides a convenient method for determining the saving accomplished by using this type of beam in place of a simple cylindrical structure. The volume V of the cylinder expressed in terms of the radius r and length l is

$$V = \pi r^2 l.$$

The volume of the beam of constant strength is obtained by rotation about the axis of symmetry and integration of elements of volume between the limits 0 and l so chosen that

$$dV = \pi r^2 dx = \pi r^2 \sqrt[3]{\left(\frac{x}{l}\right)^2}\, dx.$$

This integration yields

$$V = 0.6\pi r^2 l.$$

A comparison of the two volumes shows that the saving through use of the type of beam under discussion is 0.4 of the volume of the cylindrical beam of the same basal radius, a saving of 40%. A similar relation can be established for beams of other cross-sectional outlines.

TABLE 2

COMPARISON OF SECTION MODULI IN TERMS OF AREA FOR VARIOUS GEOMETRIC FIGURES

Geometric figure	Position of neutral axis	Section modulus Z in terms of a circle	Efficiency ratio
Square	Opposite vertices	0.209 Ar*	0.84
Hexagon	Opposite vertices	.264 Ar	1.05
Octagon	Opposite vertices	.238 Ar	0.95
Square	Center of opposite sides	.295 Ar	1.18
Hexagon	Center of opposite sides	.228 Ar	0.91
Octagon	Center of opposite sides	.258 Ar	1.03
Triangle (equilateral)	Center of gravity two sides	0.194 Ar	0.78

* Ar is the product of the area A and the radius r of the circle.

Although a beam of constant strength is as strong as a corresponding beam of constant section, its deflection under an equal bending force is 1.8 times as great.

A great many plant stems approach the form of a beam of constant strength. The stems of monocotyledons are in many cases almost perfect examples. There is no phylogenetic significance, however, to this general form, since it oc-

curs in various orders and families without any general observable pattern.

Columns

Since plant stems support loads the lines of action of which are parallel to their long axes, they must act as columns as well as beams. These may act as short columns or long columns depending upon length-diameter ratio (the slenderness ratio of mechanics).

A short column is usually considered one whose length-diameter ratio is in the order of 11 or less. The general equation for deformation of a short column is simply an expression of Hooke's law, and, as would be expected, plants with stems of short column structure occur throughout the plant kingdom.

A type of column which enters into the structure of massive stems such as the lower portions of the boles of large trees is the column of constant strength. This is a column of such shape that it increases in cross-sectional area at the rate necessary to compensate for the increase in the weight of the column itself as additional strengthening material is required toward the bottom. An enlightening discussion of this type of column is given by Timoshenko (**15**).

In the case of long columns reference must inevitably be made to Euler's equation for a column fixed at one end and free to rotate about the other. In the case of a circular column this equation is

$$\frac{P}{A} = \frac{\pi^2 E}{4\,(l/r)^2},$$

in which P/A is the unit load, E is the modulus of elasticity of the material, r is the radius of gyration of the cross section, and l is the length of the column. The significance of this equation lies in the fact that it states that the critical oad that can be borne by such a column depends upon its length and radius as well as upon the mechanical properties of the material of which it is composed. It does not depend upon the distribution of such materials except as this distribution effects the moment of inertia of the section as a whole. A column of this type, when loaded to the proportional limit (P is then the critical load), is in a state of unstable equilibrium, and it will fail completely under the least vibration or addition to the load. As will be discussed later, this probably accounts for the liane habit in many plants.

With respect to composite sections as represented by stems with discrete bundles (fig. 4) or narrow rings of vascular tissue, an important principle is involved which suggests similarities to the condition found in reinforced columns. For its mathematical expression this principle requires a slight modification of Hooke's law to express the elastic deformation of a column made up of more than one material. In its final form such an equation states that in a composite section the load is distributed to the individual parts in direct proportion to their moduli of elasticity and their corresponding cross-sectional areas. This means, also, that the first part to fail in such a system is the one with the greater modulus of elasticity-area product.

Discussion

Mechanics of woody stems.—Since the woody plant is the ancestral prototype of the herbaceous form, some consideration should be given to the role which mechanics have played in the transition from the woody to the herbaceous condition. Many tree species approach the form of a cantilever beam of constant strength in so far as resistance to wind loads is concerned. The weights of the crown and upper stem tend to

force the lower sections of the bole into compression; hence, it tends to approach the form of a column of constant strength. It is therefore a system subjected to combined stresses (compression and flexure). Serious complications arise when any attempt is made to determine the resultant form when these two stresses are considered simultaneously. OPATOWSKI (11) experienced this difficulty when he attempted to treat the whole of a tree stem as a beam of constant strength. Attempts to treat the bole of a tree as a beam and column of constant strength simultaneously or in superposition lead to an impasse engendered by the difficulty in combining an algebraic and an exponential function. The flaring bases of large trees, however, follow the form provided by the equation for a column of constant strength so closely that it appears logical to assume that this mathematical concept is involved in such stem structures.

MECHANICS OF HERBACEOUS STEMS.—The term "herbaceous" is so often loosely applied that some limits must be placed upon its meaning. For purposes of this discussion a herbaceous stem is defined as any aerial stem which does not persist as a living organ of the plant for more than one (in some biennials, two) growing season. Under this definition the herbaceous stem is not confined to annuals but may be a recurring organ which appears each growing season from the prostrate stem, rhizome, or like organ of a perennial plant.

For clarification in discussing the evolution of the herbaceous type reference is made to BESSEY (1), from which the following is quoted:

1. Evolution is not always upward, but often involves degradation and degeneration.
2. Evolution does not necessarily involve all organs of the plant equally in any particular period, and one organ may be advancing while another is retrograding.
3. Evolution has generally been consistent, and when a particular progression or retrogression has set in, it is persisted in to the end of the phylum.

On the basis of distribution within the various families investigated, no direct or sequential series of mechanical adaptations with respect to stem structure appear to exist among herbaceous dicotyledons. Rather, similar mechanical adjustments appear to parallel one another in various and often unrelated families. In contrast to the mechanical adjustments, two well-defined anatomical modifications do appear to characterize two definite phylogenetic series to be discussed subsequently.

The combination of upward and downward evolution from a mechanical standpoint is exhibited by such lianas as *Clematis*, *Aristolochia*, *Vitis*, and *Menispermum*. In all these the liane habit may be considered as a "downward" line of evolution from a mechanical standpoint, since, except when it is fortuitously accompanied by other adaptations fitting the plant to survive as a vine, the liane habit would probably lead to extermination by suppression. Lianas appear to be stems which have become Euler columns which have "failed" because the length-radius ratio has become too large for maintenance of the upright habit. The capacity of an upright stem to support itself varies directly as the stiffness factor, which is the product of the modulus of elasticity and the moment of inertia, and inversely as the square of the length. During the transition to the liane habit the stiffness factor has decreased because of the cross-sectional increase in the size of the vessels. This has been accompanied by a concomitant increase in length until a point has been reached

where the ratio has become so small that the stem is incapable of supporting the weight of the plant, and an upright habit is no longer possible without external support.

Mechanical adaptations which increase the section modulus by the change from round to polygonal cross sections are numerous throughout the plant kingdom. The mints (fig. 2) are an outstanding example where the square section increases the ability of the stem to withstand bending stresses. Other examples are seen in *Dipsacus* (fig. 3), with a hexagonal stem, and *Sericocarpus* (fig. 4), where there is a tendency toward an octagonal cross section.

Pilea pumila (fig. 5) exemplifies an extreme upward type of mechanical evolution in a species which, from a floral standpoint, belongs in a group considered to be primitive. In *Pilea* the extreme in herbaceousness has been achieved, whether by proliferation of the ray tissue or by degradation of the cambium it is impossible to determine, although the latter is suggested in the appearance of the cross section. The vascular bundles in the lower stem, with the exception of the median web bundles, are grouped in the flanges of an I-beam type of structure. The extremely thin walls of the tissue surrounding the bundles would indicate that their tensile, and especially their compressive, properties are negligible as contributing factors to the strength of the stem. The xylem components, therefore, represent an I-beam with respect to resistance to bending stresses, with each vascular bundle in one flange equally balanced by a corresponding bundle in the opposing flange. The two strands in the web are located on the neutral axis, where the tensile and compressive stresses approach zero but where longitudinal shear is at a maximum. They also serve to resist bending in a plane at right angles to that normally produced by wind pressure alone. The opposite arrangement of the leaves on either side of the center of the web insures that under wind pressure a maximum bending moment develops about this axis of the cross section.

In *Podophyllum peltatum* extreme herbaceousness is accompanied by another "upward" mechanical adaptation. Here the vascular tissue is reduced to scattered bundles. The leaves are peltate in their attachment to the stem, an arrangement which produces an upward pull under the stress of wind pressure. This resistance to the shock-load of wind pressure forces the stem into tension parallel to its long axis, a type of stress to which the many separate, flexible strands of xylem act as superb resistors.

Examples of mechanical adaptations may be found in numerous families and genera throughout the plant kingdom. There appears to be no sequential line of phylogeny in their arrangement. A similar situation is found to hold for all the mechanical modifications found in individual genera among the plants observed in this investigation.

PREVIOUS METHODS OF ANALYZING STEM STRUCTURES.—It has been the practice among botanists to treat a hollow cylindrical arrangement of the mechanical tissue in a stem as a series of coaxial I-beams, the webs of which pass through the center of the stem. It has been shown in the section dealing with mechanical principles that this arrangement of mechanical tissue can be treated as a general system, the mathematical analysis of which is universally recognized. Any mechanical advantages which may appear with respect to this type of mechanical tissue can be explained on the basis of the ratio of the section modulus

and cross-sectional area of the tissue, as compared with various other arrangements of equal amounts of material. Thus, the increase in the size of the pith relative to the diameter of the stem as found in herbaceous types, accompanied by an increase in diameter of the mechanical tissue, has the advantage of increasing the radius of gyration without increasing the solid mass. This means economy in the production of such tissue.

It has also been customary to treat stems having discrete vascular bundles as systems of coaxial I-beams. The objection to this lies in the fact that in a majority of stems each bundle does not have a coaxial bundle of equal size diametrically opposite; therefore, the true I-beam relation does not exist in such cases. Where an uneven spacing or an odd number of bundles is found in a stem, the mechanical relation is somewhat analogous to that of a reinforced beam or column. Where the xylem is surrounded for the most part by thin-walled parenchyma, the difference in elastic properties between these two types of tissue is so great that the stresses imposed by weight of the plant or bending of the stem must be resisted by the xylem or other mechanical tissue alone. The parenchyma serves primarily to maintain relative positions within the stem. It is therefore more nearly correct to treat a circle of vascular bundles as a closed ring with inner and outer dimensions such as to give the ring a total cross-sectional area and cross-sectional moment of inertia identical with those of the bundles. The bundles alone control strength and stiffness according to the modification of Hooke's law as applied to composite sections.

HISTOLOGICAL MODIFICATIONS OF MECHANICAL ADVANTAGE.—Changes which increase the strength of the fibrous tissue or its more advantageous distribution in the stem may be considered adaptations which increase the efficiency of the stem. This is especially true if they allow for a decrease in the amount of mechanical tissue requisite for withstanding the normal stresses borne by the stem.

A striking and uniformly progressive development of such a nature appears in loss of ray tissue and vessels in the secondary xylem of the higher orders where herbaceousness is very commonly found. This makes the xylem more fibrous and hence increases its elastic resistance to stress. In this respect, even on the basis of the meager number of species studied in this investigation, two definite lines of progression appear.

One of these consists of a congregation of the rays in the secondary xylem external to the outgoing leaf traces. This is accompanied in the more advanced and more herbaceous members of the evolutionary sequence by a loss of rays in the intervening tissue and a reduction in the number of vessels in the same tracts, as may be seen in *Medicago sativa* (fig. 6). The increase in fibrous tissue strengthens the xylem masses between the parenchymatous aggregations, so that, in accordance with Hooke's law, less xylem is needed to achieve mechanical stability. In consequence, the stem becomes more herbaceous as the secondary growth becomes smaller in amount owing to early termination of the growing period.

This is the type of evolution of the herbaceous stem propounded by JEFFREY (**7, 8, 9**). This trend seems quite definitely to follow from the Ranales, through the Rosales, Celastrales, Umbellales, and Rubiales, and to reach its culmination in the Asterales.

The other line of progression consists of the attainment of herbaceousness by a thinning of the vascular cylinder, but

without ray congregation. Increase in strength and stiffness is accomplished by a loss of ray tissue and vessels in those regions of the stem where the xylem content has been reduced by reduction in cambial activity. This type of evolution appears to occur at intervals through the Caryophyllales and Polemoniales and to reach its culmination in the Scrophulariales and Lamiales. This development is accompanied by a peripheral modification of the cross section which increases the magnitude of the section modulus, a modification not observed in the parallel line discussed above. The members of the Solanaceae and Boraginaceae display a definite tendency toward the hexagonal stem cross section which is superior, in so far as the section modulus is concerned, to the circular type. This assumption is conditioned by a proper distribution of the leaves, usually inserted at opposite vertices of the hexagonal cross section. In the Labiatae and Verbenaceae the specialization appears to have progressed even further, and the stems have a square cross section with opposite leaves attached to the flat faces. This insures that the major bending moment will develop about the median axis perpendicular to opposite faces, which is the position guaranteeing the maximum moment of inertia in this type of section.

This line of evolution is that suggested by SINNOTT and BAILEY (**13, 14**).

Although this does violence to the phylogenetic relations as laid down by ENGLER and PRANTL, it is in close accord with the systems proposed by BESSEY (**1**) and HUTCHINSON (**6**).

Mention has already been made of the inclusion by HUTCHINSON of the genus *Phryma* in the Verbenaceae and that this appears to be justified on an anatomical basis as well as upon the floral basis by which the monotypic family Phrymaceae thus became a part of the Verbenaceae.

Another arrangement by both BESSEY and HUTCHINSON also appears to be justified on the basis of anatomical considerations as well as others. This is the derivation, at least in part, of the Compositae from the Umbellales. In the lower composites schizogenous oil ducts are commonly found which are associated with the phloem, a condition which disappears in the higher members of the family. In the Umbelliferae schizogenous oil ducts also occur, not only associated with the usual external phloem, but also associated with what appear to be remnants of internal bundles consisting only of phloem and of small amounts of surrounding supporting tissue. These appear to be vestiges of the internal bundles, so striking in the Araliaceae, in which the phloem has in part persisted and become the tissue which is responsible for the development of longitudinal oil ducts. Hence, schizogenous oil ducts appear to furnish a phylogenetic link among the members of these groups paralleling the evidence furnished by the floral structure.

Summary

1. Eighty-two species, representing forty-one families of plants, characterized, at least in part, by herbaceous stems, were examined to determine the role of mechanics in the evolution of the herbaceous plant stem.

2. There appears to be no definite and sequential line of evolution which can be based on mechanical adaptations in stem structure for the herbaceous type. Many mechanical adaptations occur in primitive genera as well as in the higher groups, and in many of the higher herbaceous orders there are practically no striking structural modifications which indicate an increase or decrease in the mechanical efficiency of the stem.

3. Lianas are plants which have been able by various other adaptations to survive in spite of a habit which is characterized by a length-diameter ratio so large that the mechanical tissues are incapable of supporting the stem without external aid.

4. Stems which at maturity reach a length-diameter ratio at which they approach the form of an Euler column will probably be scrambling, climbing, or prostrate in habit. The stem's ability to support itself is probably doubtful at a length-diameter ratio of 60 plus, and practically impossible at 100 plus.

5. Massive stems, such as the boles of large trees, tend to assume the form of a column of constant strength near the base, a tendency which disappears with reduction in massiveness during the course of evolution.

6. Certain polygonal cross sections in stems, although following no special phylogenetic sequence, represent adaptations which increase the mechanical efficiency of the stem in many cases; others, such as triangular cross sections, may produce the opposite result.

7. Discrete bundles and thin rings of vascular or mechanical tissue in various species, while achieved by different phylogenetic sequences, are essentially similar from a mechanical standpoint when viewed in the light of Hooke's law as applied to composite sections.

8. Two lines of evolution in herbaceous stems are indicated: (*a*) proliferation of the ray tissue and its congregation into large radial tracts, thereby breaking the xylem cylinder into separate bundles, with an accompanying decrease of rays and vessels in any vascular tissue intervening between the bundles, and (*b*) thinning of the xylem cylinder through a decrease in cambial activity which is accompanied by a loss of rays and a reduction in the number of vessels in the regions so affected.

9. HUTCHINSON's inclusion of the genus *Phryma* in the Verbenaceae is given added support by the similarity in stem anatomy of the genus to that of certain members of the Verbenaceae.

10. The derivation, at least in part, of the Compositae from the Umbelliferae, as suggested by BESSEY and HUTCHINSON, is supported by the anatomical similarities relative to the schizogenous oil ducts which are found in both groups.

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MONOLITH METHOD OF ROOT-SAMPLING IN STUDIES ON SUCCESSION AND DEGENERATION[1]

J. E. WEAVER AND JOHN W. VOIGT

Introduction

A new method has been devised by which a complete sample of an entire root system may be taken from soil surface to maximum depth of penetration. The roots are separated from the soil without injury to them or displacement of individual roots from their natural position. They are examined in the laboratory in relation to the various horizons of the soil profile. Monoliths 12 inches wide, 3 inches thick, and 3–5 feet in depth are taken from the walls of trenches made in selected pure stands of each species. Roots are obtained from the monolith by a system of soaking and gentle washing. A special technique is used in mounting. Lighting for photographing is by electro-flash units. The root system is sectioned so as to obtain the oven-dry weight for each 6 inches or foot in depth as well as for each major soil horizon. A brief description of the method may be found in *Science* (**7**) and a complete one in *Ecological Monographs* (**8**).

This new method, which permits the studying of the intimate relations of roots and soils and of measuring root production quantitatively at various soil levels, has been used extensively. Descriptions have been made of the profiles of twenty soil types, from which thirteen species (and about fifty monoliths) have been taken. The chief purpose of these studies was to examine soil-root relationships in various soil types (**8**). The present paper is concerned with results obtained by a modification of the method and its use in studying succession and degeneration of vegetation.

[1] Contribution from the Department of Botany, University of Nebraska, no. 154.

Observations

Modification of method

A modification of the original method consisted in increasing the width of the monolith to 2.5 feet, so that a more continuous view of the underground plant parts might be obtained. This necessitated obtaining the soil and roots in sections 3 feet long and 3 inches thick where roots extended deeply. The lower section was taken from the vertical wall of a trench, directly below the upper one. To prevent the soil at 3 feet depth from crumbling when the bottom of the upper frame holding the monolith was fitted in place,

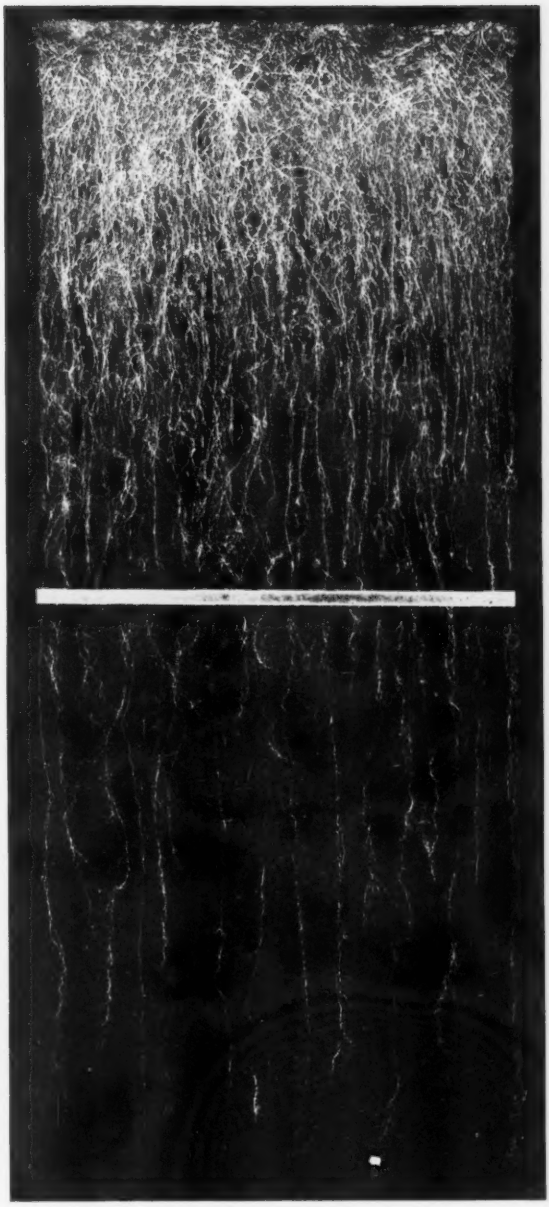


FIG. 1.—Root system of *Andropogon furcatus* taken from the two sections of a monolith, each 2.5 feet wide, 3 inches thick, and 3 feet long. White strip covers 37th inch, which is lost in obtaining sample. Note gradual decrease in density of root mat with depth and very uniform distribution of roots at any level.

a straight-edged board was placed horizontally against the trench wall at exactly 37 inches in depth and fastened securely in place while the upper monolith was being removed. Thus, only 1 inch of soil and roots was lost where the soil was removed to make room for the lower end of the upper monolith frame. In mounting the roots and in the photographs, this space was occupied by a thin strip of wood (fig. 1).

FIG. 2.—Portion of root system shown in figure 1. Roots are natural size, and branches are in normal position; taken at depth of 24–29 inches.

REPLACEMENT OF BIG BLUESTEM BY KENTUCKY BLUEGRASS

On an area of low, nearly level, but well-drained land near Lincoln, Nebraska, an excellent bluestem prairie occurred. This was the 50-acre tract about 3 miles northeast of Havelock, a suburb of Lincoln, which was intensively studied by WEAVER and DARLAND over the period 1943–1946, inclusive (**6**). A chief dominant in this rich, alluvial Wabash silt loam was big bluestem (*Andropogon furcatus*). It frequently occurred in local pure stands of sod. The foliage normally attained a height of 3 feet, but the coarse flower stalks were often 6–7 feet high The uniformity of the grass above ground was no more marked, however, than that of the coarse roots in the soil (fig. 1). Although there was some crisscrossing of roots in the upper soil, the general direction was vertically downward. The upper or A horizon (0–24-in. depth) contained the bulk of the root mass. In the subsoil (C horizon—24–41 inches) the roots decreased rapidly in number; but many extended deeply, even to 6 feet (fig. 1).

This wonderfully developed root system loses much in detail in photographing it in its entirety. Detail of the root pattern is shown in figure 2. The coarse main roots are 2–3 mm. in width. The glistening white stele may be seen through the somewhat transparent cortex.

Decrease in root weight with depth was very marked but was about normal for this species. In the surface 4 inches the roots themselves often furnished about half the total weight, rhizomes and stem bases in the soil being, as usual, about equally heavy (**9**). Despite the small amount of the lower half of the root system (only 2.8% of the whole), this

portion was undoubtedly active in absorption far out of proportion to its weight. During drought it enabled this species to remain alive where numerous less deeply rooted grasses died (5) (table 1).

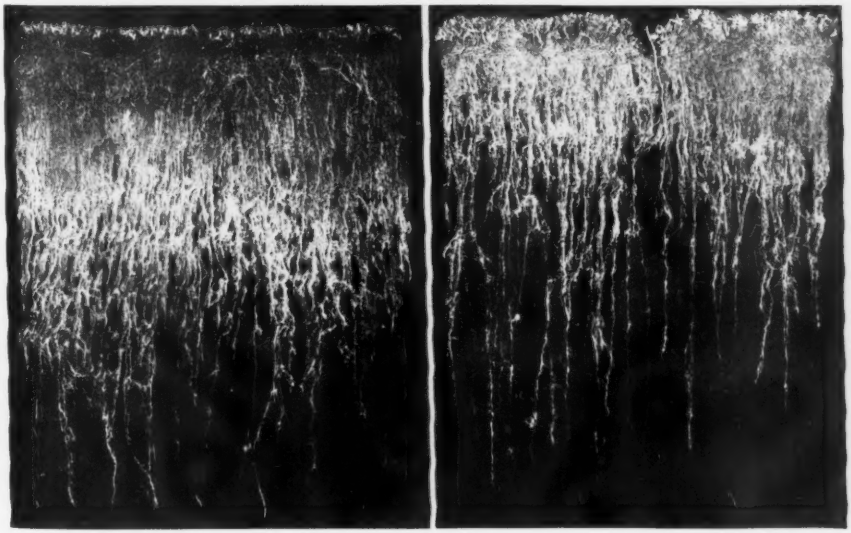
As a result of grazing this prairie, big bluestem decreased in vigor, waned, and died, and like other prairie grasses it was replaced by a nearly continuous sod of Kentucky bluegrass (*Poa pratensis*). This occurred during a period of only a few years (1943–1946) and was the result of too early grazing in spring and overstocking (6). The roots of a representative sample of the underground parts of bluegrass were secured for study. The great bulk of the root mass (89.9%) occurred in the surface foot of soil and nearly all the remainder in the second foot. Only rarely did a root end extend beyond 3 feet in depth (fig. 3). The roots are very fine and draw intensively upon the surface soil for moisture and nutrients. Their distribution and weight at the several depths are shown in table 1.

THE SOIL PROFILE.—The remarkably uniform distribution of the roots of big

TABLE 1

OVEN-DRY WEIGHT OF ROOTS AND PERCENTAGE OF DRY WEIGHT AT SEVERAL SOIL DEPTHS

DEPTH (IN.)	ANDROPOGON FURCATUS		POA PRATENSIS	
	Wt. (gm.)	% wt.	Wt. (gm.)	% wt.
0– 6	62.87	69.0	42.55	76.5
6–12	12.59	13.8	7.44	13.4
12–24	9.79	10.7	4.81	8.7
24–36	3.38	3.7	0.81	1.4
36–48	1.23	1.4	.....	.....
48–60	0.80	0.9	.....	.....
60–72	0.49	0.5	.....	.....
Total	91.15	100.0	55.61	100.0



FIGS. 3–4.—Fig. 3 (*left*), root system of *Poa pratensis* showing relatively shallow depth to which roots penetrate and concentration of mass of roots in surface soil. Fig. 4 (*right*), root development of *Bouteloua gracilis* (*left*) and *P. pratensis* (*right*) in old, upland pasture near Lincoln, as shown in monolith 3 feet deep. Total root systems weighed only 28.2 gm., 82% of which occurred in surface 6 inches of soil.

bluestem to great depths is surprising when the soil profile is examined. This Wabash silt loam is an alluvial soil.[2] It is also a young soil without horizon differentiation to a depth of 41 inches, except the accumulation of noticeable amounts of organic matter to a depth of 24 inches. Below a depth of 41 inches there is an X layer or buried soil. It has horizon development comparable with mature soils. The X layer represents material of a different age and deposition from that in which the present soil at the surface has developed. It has undergone some modification since it was buried by the younger material (perhaps 150 years ago), but the several horizons are clearly defined. The lower, XB_3, horizon is a strongly developed horizon of lime carbonate accumulation.

The horizons are as follows:

A	0–24 in.	Surface soil
C	24–41 in.	Subsoil or parent material of the present soil
XA_1	41–50 in.	Surface soil of buried soil
XB_{1-2}	50–66 in.	Leached subsoil of buried soil
XB_3	66–72 in.	Strongly calcareous subsoil of buried soil

Horizon	Depth (in.)	Description (color is that of dry soil)
A_{1-1}	0– 1	Dark gray, friable, platy mulch silt loam; pH 6.5
A_{1-2}	1– 6	Dark gray silt loam; friable; granular; pH 6.5
A_{1-3}	6–24	Grayish-brown silt loam; friable; coarse; granular or nuciform; pH 6.5
C_{1-1}	24–34	Light brownish-gray, friable silt loam; somewhat stratified with darker lenses; irregular medium blocky; pH 6.5
C_{1-2}	34–41	Same color as layer above, but silty clay loam showing a little more stratification; pH 6.5. The strata in this layer are separated by a film of light gray silt and very fine sand
XA_1	41–50	Black clay; large blocky; plastic when wet; hard when dry; pH 6.5
XB_1	50–60	Grayish-brown, blocky silty clay; moderately hard when dry; moderately plastic when wet; pH 7.0
XB_2	60–66	Grayish-brown, silty clay loam; blocky; moderately hard when dry; moderately plastic when wet; pH 7.0
XB_3	66–72	Light yellowish-brown, nuciform, heavy silt loam; friable; calcareous, including streaks and seams of segregated lime carbonate; pH 7.5

[2] The soil profile was described by B. H. WILLIAMS of the Division of Soil Survey, U.S. Department of Agriculture, and the Conservation and Survey Division, University of Nebraska.

The rich A horizon of the buried soil seemed to have no visible effect upon root development or distribution. Where the new soil is shallower, root development of certain grasses has been found to be greater in the old topsoil.

SIGNIFICANCE OF CHANGE.—The effects of the degeneration of climax prairie into bluegrass pasture have been ascertained aboveground. The change from prairie to bluegrass was a sign both of degeneration and of decreased forage production. The annual yield under grazing was obtained by measuring the monthly yield from early spring until late fall. This was done by means of large portable exclosures. The prairie grasses, among which big bluestem was most abundant, produced 39–99% more forage than bluegrass. Moreover, bluegrass is a less palatable and less desirable pasture grass, partly because it often dries and is more or less dormant in midsummer.

It was in one of the exclosures, left permanently in place since 1946, that the sample of big bluestem roots was taken

in June, 1949. The bluegrass was secured outside the exclosure at the same time.

Knowledge of the root systems adds much to an understanding of the ecology of these grasses and the thousands of square miles of pasture lands which each occupies. Only the upper portion of the soil is utilized by bluegrass. Often it is more shallowly rooted than in this pasture; only rarely is it deeper. During the dry climatic cycle of 1934–1940 in the Midwest, practically all bluegrass pastures of eastern Nebraska succumbed to drought. They remained intact only along streams where the water table was high. In Iowa, also, great damage occurred. Extensive soil sampling during the drought revealed that the soil under bluegrass had much available water below 2.5 feet. With such subsoil moisture and the nutrients of deep, rich, virgin soils, big bluestem maintained life and often thrived, while the more shallowly rooted bluegrass died.

Native bluestem pastures often almost exclude the invasion of weeds until the grasses are largely replaced by bluegrass. Then the entrance of invading ruderals is rather easily accomplished. Once they extend their roots below the 2-foot level, competition underground is greatly decreased. It is then that the ragweeds (*Ambrosia artemisifolia* and *A. psilostachya*), ironweed (*Vernonia baldwini*), and a host of other species thrive. Since 1946 ironweed and buckbrush (*Symphoricarpos occidentalis*) alone have spread so widely and are so densely aggregated in this experimental pasture that the yield of bluegrass has been decreased by about one-third.

Subseres in upland pasture

Bluegrass pastures represent a third or fourth stage in the degeneration of true prairie under injudicious management. It is a common pasture type. But as one approaches the western edge of true prairie, as at Lincoln, blue grama (*Bouteloua gracilis*) often takes the place of bluegrass or, frequently, shares the area with it. The root relations of these grasses in Carrington silty clay loam are shown in figure 4. Development of blue grama is scarcely better than that of the bluegrass, although the roots do penetrate somewhat more deeply. Both species occurred as isolated patches with bare soil between, except for the part occupied by low-growing pasture weeds. These grasses grew in an old soil developed on glacial drift. It was very compact. The dark grayish-brown, granular, heavy silty clay loam of the A horizon extended to a depth of only 7 inches. The subsoil of similar color and texture (B horizon) was only 15 inches deep. Below the solum (22–36 in.), the parent material consisted of dark yellowish-brown clay with a blocky structure loosely filled with small rocks and pebbles. Only a few of the longest roots of blue grama extended to a depth of 3 feet.

This root development of these grasses on upland is not unusual. Nor is the replacement of the deeply rooted bluestems and other climax grasses by more shallowly rooted ones uncommon. In a native pasture of 210 acres near Lincoln, three distinct pasture types or stages in degeneration are clearly evident. They have resulted partly from distance to the water supply, which is near the southern end of the mile-long pasture, but also from the lesser accessibility to the northern end of the pasture because of steep slopes (9–11%) and deep ravines. This distant portion is, after 46 years of grazing, still near the prairie stage. Conversely, the southern part, on similar rolling hills, has been reduced to a low-grade subsere dominated largely by bluegrass

and blue grama. An intermediate stage with about half bluestems and half bluegrass occupies the central part of the pasture. The Carrington silt loam is fairly uniform throughout, but it is deeper than the soil type just described.

Monoliths of soil were taken in representative areas of each grade of pasture. In the high-grade type the depth of the dominant little bluestem (*Andropogon scoparius*) was 42 inches, and the total root weight was 84.3 gm. In the mid-grade samples the weakened little bluestem was 36 inches deep and Kentucky bluegrass 24 inches. Combined root weight was 68.4 gm. When the bluegrass and blue grama were sampled in the low-grade pasture, where all prairie grasses had disappeared, the depth of bluegrass was again 24 inches, that of blue grama was 32. Dry weight of the root sample was only 35.1 gm. Thus, if the root weight in high-grade pasture is considered 100% (although even here the roots were not so heavy as those of ungrazed prairie), those in the mid-grade were 81% as heavy. Those in the bluegrass–blue grama subsere had been reduced to 42%.

Root distribution between bunches

Among such sod-forming grasses as buffalo grass (*Buchloe dactyloides*) and western wheat grass (*Agropyron smithii*), a monolith 12 inches wide gives one accurate information on the general appearance and the interrelationships underground. But where monoliths 12 inches wide are taken directly beneath a bunch of little bluestem, prairie dropseed (*Sporobolus heterolepis*), or other bunch grasses, there is still some doubt as regards amount of root materials between the bunches. In earlier work, where the trench and hand-pick method was employed (1–4), information on direction and extent of lateral spread was recorded. But this gave no quantitative answer to the question. Before the 30-inch-wide monolith was employed, all samples of roots were obtained directly below the bunch.

This problem has now been solved, and quantitative data have been obtained for little bluestem and certain other prairie grasses. Root distribution in a pure climax stand of little bluestem in Sharpsburg silty clay loam near Lincoln is shown in figure 5. The sample for the monolith was selected upon the basis of an approximately normal distance between bunches. The background and foreground where the monolith was taken had approximately the same distribution of bunches as the upper surface of the monolith in figure 5. In the sample the several bunches at the soil surface occupied 12 of the 30 inches of width, and interspace between bunches the remaining 18 inches. The root mat was cut vertically, so that all root material in the 2 feet below the bunches could be weighed separately from that between them. Since the stem bases and short rhizomes were concentrated directly beneath the bunch in the surface soil, the upper 3 inches of the entire mat was discarded. Moreover, the root mass was cut horizontally at a depth of 12 inches so as to separate all material in the second foot from that in the first. Maximum lateral spread of the roots beyond the outer edge of the crown was 5–12 inches; it was often as great and sometimes greater than that in the surface 3 inches of soil.

Total oven-dry weight of material under the bunches at the 3–12-inch depth was 10.98 gm. or 0.92 gm. per inch in width of the 3-inch-thick mat. Between the bunches it was 14.98 gm. or 0.83 gm. per inch. At the 12–24-inch level the total weights were 0.48 and 0.42 gm. per inch,

respectively. Some roots, of course, entered the back of the monolith, and others penetrated into the front surface. According to these results, the upper soil layer in the interbunch areas (3–12 in.) was 90% as well occupied by roots, and the second foot 87% as well filled, as that portion directly beneath the bunches.

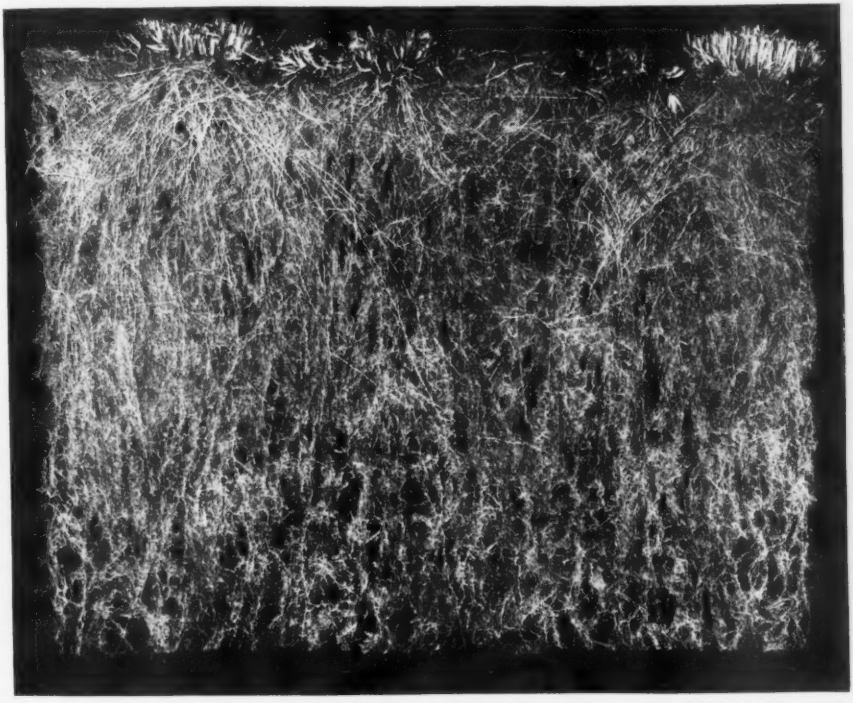


FIG. 5.—Roots of *Andropogon scoparius* in monolith of soil 30 inches wide, 3 inches thick, and 2 feet deep, taken from climax true prairie.

A monolith of similar dimensions was taken in a practically pure stand of prairie dropseed only a little distant in the same soil type. This is a grass of even more pronounced bunch habit than little bluestem. The dense mat of long, outstretched leaves forms a continuous mulch between the bunches, the crowns of which are elevated 1–3 inches above the soil surface. This habit of growth occurs even under mowing and may account in part for the paucity of interstitial species in pure stands of this dropseed. Two bunches, 8 and 4 inches in width, were 10 inches apart in the monolith. The remaining surface was mostly without other vegetation. Examination showed that some roots spread 5 inches from the bunch in the surface inch of soil; at 2 inches depth many spread 5–7 inches. Weight of roots per inch between the crowns of the bunches at 3–12 inches depth was 82% as great as that directly beneath the crowns; in the 12–24-inch depth it was 108% as great as that beneath the bunches.

One may conclude from these and similar data that in stabilized true prairie, where interstitial species of grasses and forbs occur between the bunch grasses, the density and weight of the underground parts are quite uniform. It is believed that this varies scarcely more in uniformity than the root masses under stands of sod-forming grasses. But this uniformity of root distribution does not occur generally in subseres, even those in advanced stages of development.

Competition underground in stages of succession

One part of the prairie near Lincoln, used for ecological studies for many years, is a subsere resulting from a quarter-century of grazing. Livestock have now been excluded from this portion for a period of 11 years, and the vegetation is nearly in a prairie climax. One chief difference is the relict patches of Kentucky bluegrass which still exist, although surrounded by big bluestem. In late summer, despite the excellent development of bluegrass, these areas appear as depressions in the 3-foot foliage cover of big bluestem. The underground relationships of these species were examined in June by means of wide monoliths (fig. 6).

The density of the roots in this very deep, rich, Judson silt loam was greater even than that described earlier from Wabash silt loam. This soil with a 20-inch-deep A horizon is developed in silty colluvium. It lies between a soil type developed from loess on the upland and Wabash silt loam of the bottomlands. The wonderful network of bluegrass roots was 24% heavier than that from a similar soil volume in the Wabash soil. Those of big bluestem were 34% heavier. The network of bluegrass roots rapidly became thinner below 2 feet; a few roots were 4 feet deep. But some roots of big bluestem penetrated into the sixth foot of soil.

Aboveground the overtowering big bluestem cast a dense shade. Only 1–3% of light reached the soil beneath its foliage, and no bluegrass occurred there. At the edge of the bluestem sod, bluegrass received light of low intensity, and where the tall grass lodged over the bluegrass the intensity was often reduced to 1% or less. Examination of figure 6 shows that, although no bluegrass roots occur beneath the big bluestem, some of the roots of the bluestem have entered the soil under the bluegrass sod. In fact, there seemed to be a tendency for many of the roots to turn away from the soil area directly below the bluestem sod (which extended many feet to the right of fig. 6) and grow toward the area of bluegrass.

Following this clue, careful examination of root spread was made at many points along the tension lines between the two species. Invasion of big bluestem into the root mat of bluegrass occurred frequently and was often pronounced (fig. 7). Root behavior of switchgrass (*Panicum virgatum*) was then studied. It was found to invade adjacent patches of bluegrass in a similar manner (fig. 8). It is of interest that in both of these species invasion by lateral spreading of roots, especially in the upper 2 feet of soil, was 12–24 inches in advance of the crown, the spread of which was determined by development of rhizomes.

Nearly all the previous studies of roots of native species made by the senior writer and his students were in nearly stabilized or climax vegetation where plant populations were dense. Although the lateral spread of the roots was examined, measured, and recorded, it was usually not found to be great, and the plant, especially grass, presented a very

symmetrical pattern. This was probably because of a rather uniform habitat resulting from competition for water and nutrients. For example, twelve to eighteen separate lots of big bluestem and switchgrass had been studied in various habits. The wide monolith offers a means not only of presenting clearly differences in root distribution but it is also a quantitative method of expressing these differences. Sometimes the cause of the success of a particular species in

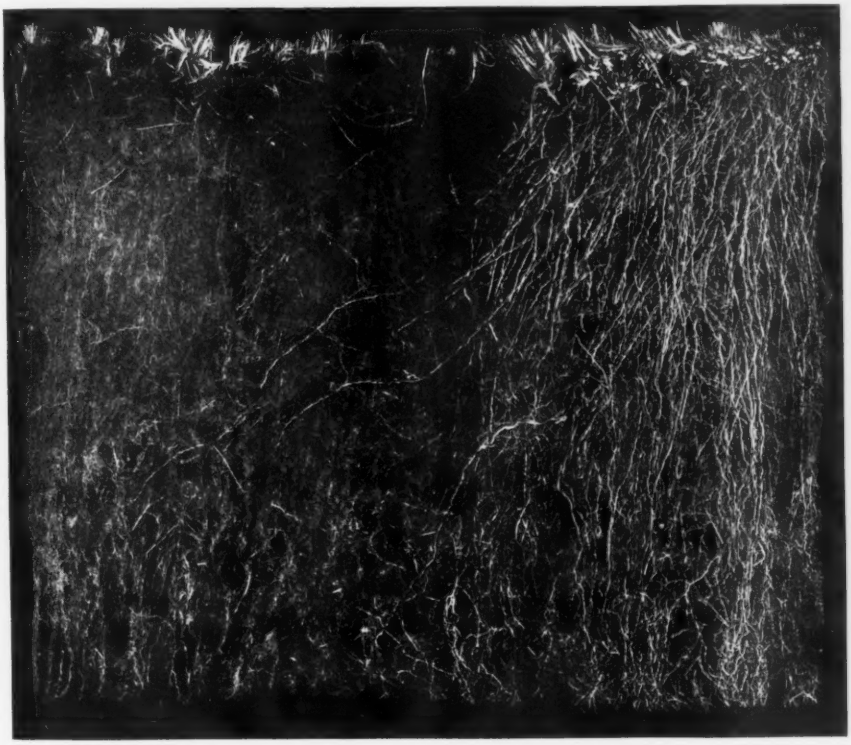


FIG. 6.—Roots of *Poa pratensis* (*left*) and *Andropogon furcatus* (*right*) from monolith 2 feet deep in Judson silt loam. Note great difference in fineness of roots and tendency of some big bluestem roots to spread downward and outward under bluegrass.

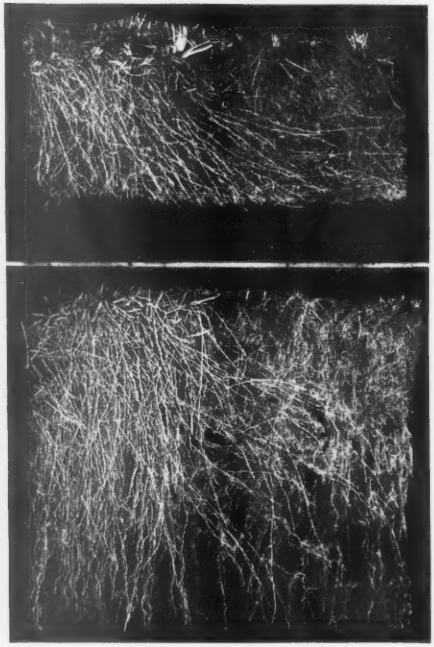
soils, but not one exhibited a root behavior such as has here been recorded in subseres in two very different soil types. Exceptions to this uniformity of pattern were found, of course, in thinly populated areas such as gravel slide and shifting sand and on much-eroded loess hills.

It would seem clear from these findings that much is to be learned in a study of succession by an examination of the root competition and its role in succession may be traced to adjustments of the root system to soil horizons at considerable depth.

ADJUSTMENT OF ROOT SYSTEM TO SOIL CONDITIONS

The soil has marked effects upon the root habits of plants (**8**). This is especially noticeable where the vegetation

develops upon soils with claypans. The preceding examples are of roots developed on zonal soil types in Prairie soil. But intrazonal soils also occur in places—soils with one or more horizons overdeveloped and in marked contrast to the "normal" or zonal soil. Small areas of such soils as Crete silty clay loam with a marked claypan occur on certain hillsides in grassland adjoining the experimental prairie 3.5 miles northwest of the University of Nebraska. Such areas, widely scattered in the Prairie and Chernozem soil zones in eastern Nebraska, are of much interest from the viewpoint of plant succession. Many of them were clothed with bluestems before the great drought (1934–1940); but during the widespread invasion which followed the drought, western wheat grass moved into these places following the death of the bluestems. This grass seems to be better adapted to these soils than any other species of the true prairie, for, despite many years of normal or above-normal rainfall, it prevails in nearly pure stands against all invaders.



FIGS. 7–8.—Fig. 7 (*above*), roots from block of soil 17 inches wide, 3 inches thick, and 7 inches deep, showing lateral spread of roots of *Andropogon furcatus* far into territory occupied at soil surface by *Poa pratensis*. Fig. 8 (*below*), roots of *Panicum virgatum* (*left*) and *Poa pratensis* (*right*) from monolith 2.5 feet wide and 2 feet deep. Note wide spreading of coarse roots of tall *Panicum* into area of bluegrass far beyond rhizomes in surface soil.

Because of the great difficulty in securing samples in claypan soils, a monolith 12 inches wide was used. Wheat grass has very long roots, often 7–8 feet in extent. For the purpose of showing the essential features in root-soil relations, however, only 6 feet of the root system was required (fig. 9). The photograph is large enough to show much detail, and the root system, excavated in June, 1949, is typical of many others from soils with claypans.

Root development was similar to that in a zonal soil in the first 13 inches or A horizon. The soil was mellow and granular in structure and nearly neutral in reaction (pH 6.8). Both water and roots penetrated easily. The 480 main roots and their branches formed a dense mass.

At 13 inches depth there was a very abrupt transition to a blocky prismatic structure with a pH value of 6.6. Clay content increased rapidly with depth, and the soil was extremely hard when dry. Vertical prisms 3–4 inches in diameter were often five- or six-sided and 8–12 inches long. They were sometimes broken by somewhat horizontal cracks. In digging the trench, this B horizon (13–28 in.) was removed only after much labor.

Roots were distinctly fewer in the B horizon. Only about 150 entered this claypan, and the number decreased to 68

FIG. 9.—Two sections of *Agropyron smithii* 12 inches wide taken to 3 feet in depth (*left*), and 3–6 feet in depth (*right*). Note dense mat of roots from granular soil of A horizon (0–13 in.) and more open root mat from claypan or B horizon (13–28 in.). In loessal soil of C horizon (below 28 in.) root branching and weight of roots greatly increased.

at 2 feet in depth. Moreover, branching of the roots was less pronounced in this horizon. Many of the roots penetrated the compact soil vertically through the columns (probably when they were quite moist), but they were also common in the crevices between them. Here they branched abundantly but mostly in the plane of the crack.

Beneath the solum in the C horizon of parent material the soil was both mellow and moist, and root branching greatly increased. This was below 28 inches. The yellowish loess, which was a silty clay loam, had a much smaller content of clay and a massive structure. There was an abundance of lime, often in small concretions. The pH value was 7.5. In this loessal horizon a third type of root distribution different from that in the A or B horizons accompanied the change in soil environment. The roots were often flattened on the faces of the small blocks, which cleaved in all directions. Thus, branching occurred in all planes. Although some of the cloddy masses were penetrated by roots, they were far more abundant on their surfaces. When this moist, soft loess was washed away, there remained a glistening white mass of roots with branches running out at all angles (fig. 9). Below a depth of 4 feet the parent loess contained more silt and less clay than above. This massive, friable loess, with vertical cracks every few inches, had no definite horizontal cleavage. The main roots followed the crevices where thin films of organic colloids had collected.

Claypan soils are not receptive to water. When dry, the shrinking of the clay results in deep, wide fissures through the claypan layer. It seems certain that much moisture is supplied to the C horizon by water running down these cracks. Some of this water also wets the bottom of the claypan layer as well as the parent material. Roots of bluestems and most other grasses cannot successfully penetrate through well-formed claypans in this area and hence do not thrive. Conversely, roots of wheat grass become adjusted to these soils. Often greater root development occurs below the claypan than within it.

In the present example root weight in grams at 0–6 and 6–12 inches and then by feet was 10.74, 2.61, 2.17, 2.27, 1.58, 0.78, and 0.56. This shows only a slight increase of materials in the third foot. But if the root weight at 16–28 inches is compared with that at 28–40 inches, there is an increase of 32% in weight in the upper foot of mellow loess soil over that in the lower foot of the claypan. Such differences have never been found by the writer except in other samples of wheat grass in claypan soils.

Summary

1. A modification of the new, monolith method of securing complete samples of an entire root system is illustrated. Blocks of soil 2.5 feet wide, 3 inches thick, and 3–6 feet in depth are taken from a vertical wall of a trench. The method permits a study of the intimate relations of roots and soil and of measuring root production quantitatively at various soil levels.

2. The roots of *Andropogon furcatus* were very heavy in climax prairie on Wabash silt loam (91.1 gm. per sample). They were uniformly distributed, decreased gradually in weight with depth, and extended downward 6 feet.

3. When *Poa pratensis* replaced *Andropogon furcatus* after several years of overgrazing, its root system was relatively light (55.6 gm.), entirely confined to the surface 3 feet, and had 89.9% of its

weight in the surface foot of soil. In a similar pasture of *P. pratensis* and *Bouteloua gracilis* in (upland) Carrington silty clay loam, root weight was still less (28.2 gm.), and roots of *P. pratensis* were only 2 feet deep. Thus, susceptibility of bluegrass pastures to drought and to invasion by deeply rooted weeds is more readily comprehended.

4. Root distribution in bunch-grass prairie was remarkably uniform below a depth of 3 inches and not unlike that under sod-forming species. Root weight was only slightly concentrated beneath the bunches.

5. The broad monolith method often revealed the presence of widely spreading roots of *Panicum virgatum* and *Andropogon furcatus* at depths of 3–24 inches below adjacent stands of *Poa pratensis*. This indicated invasion of territory 12–24 inches horizontally beyond that shown by the top and rhizomes in the surface soil. Such wide spreading of roots had not been recorded before in studies of native grasses in climax prairie.

6. Adaptation of the roots of *Agropyron smithii* to soils with claypans illustrates three distinct environments underground and remarkable soil-root relationships. A granular A horizon of mellow, moist soil resulted in a well-branched, heavy, normal development of roots. In the blocky, prismatic, very compact claypan beneath, roots were fewer, branching was poor, and roots penetrated the soil with difficulty. In the mellow, moist soil below the claypan, root branching greatly increased, occurred in all planes, and total weight of roots was a third greater in this more favorable environment. Such soils affect normal succession, since many climax species cannot thrive where they occur.

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SOME EVIDENCES FOR THE INTERACTION OF TAPETAL AND SPOROGENOUS CELLS IN CERTAIN VASCULAR PLANTS

W. N. STEIL

Introduction

It is well known by botanists that the tapetum in vascular plants is digested by the spore mother cells and the developing spores and hence serves as food for these cells.

The behavior of tapetal cells in both homosporous and heterosporous vascular plants has been studied by a number of workers, including BEER (1) in *Helminthostachys zeylanica*, TISCHLER (28) in *Ribes hybridum*, BURLINGAME (6) in three genera of the Ophioglossales, STEIL (22–25) in *Ophioglossum vulgatum* and *Botrychium virginianum*, COOPER (8) in some angiosperms, including *Lilium*, ROEVER (19) in *Hosta caerulea*, SMITH (20) in *Galtonia candicans*, CHRYSLER (7) in *Ophioglossum palmatum*, and WITKUS (30) in *Spinacea oleracea*.

The following tapetal phenomena have been described by these workers: amitosis, cell and nuclear fusions, the formation of binucleate and quadrinucleate cells, incomplete mitosis and incomplete cell division, and, in the Ophioglossales, the formation of a nutritive multinucleate plasmodium which may occupy a considerable portion of the sporangium.

It has been shown by the writer (22–25) that incomplete mitosis and not amitosis is a characteristic tapetal phenomenon in two genera of the Ophioglossales. This observation has been confirmed by CHRYSLER (7) in *Ophioglossum palmatum*.

These peculiar incomplete divisions were first described (21) in the sporogenous cells of the apogamous *Nephrodium* (*Dryopteris*) *hirtipes*. Later DÖPP (9–12) discovered the same phenomenon in some other apogamous ferns, and more recently Sister MARY THOMASINE (27) described incomplete mitoses in the apogamous *Polystichum tsussemense*.

These nuclear divisions, as has been stated elsewhere (26), are like those which have been experimentally produced and described as interrupted divisions by a number of investigators (5, 17). Nuclear divisions described as endomitotic by other workers (3, 4, 13, 14, 15, 18, 30) may also be considered as incomplete nuclear divisions.

The incomplete divisions are the result of interruptions of the mitotic process and may be caused by chemical agents such as colchicine, by different kinds of radiation, and by low and high temperatures. Since some of the interrupted divisions are completed, it is obvious that these expressions (incomplete and interrupted) are not synonymous. In an incomplete mitotic division the chromosomes are split in the usual manner, and their number is doubled, but they are not distributed between two daughter nuclei.

This investigation is concerned with the probable interaction of tapetal and sporogenous cells in some pteridophytes and in an angiosperm. Such a relationship of the two kinds of cells in the sporangia of vascular plants has not heretofore been reported.

Material and methods

Small portions of the spikes of *Botrychium virginianum* and *Ophioglossum*

vulgatum and of the fronds of *Nephrodium hirtipes* in various stages of development were fixed in Flemming's medium solution, washed in running water, dehydrated with ethyl alcohol, infiltrated with xylol, imbedded in paraffin, and sectioned 8 μ in thickness. Young and older anthers of *Lilium longiflorum* var. *insulare* were cut into small pieces at right angles to their length and fixed in Flemming's medium or in Navashin's solution. The material was then imbedded in paraffin and sectioned 8 μ or 10 μ in thickness. To insure better penetration of the fixing solutions, the air was evacuated immediately after the material was put into the fixing fluids.

Some of the sections were stained with iron haematoxylin alone, and others were counterstained with fast green. Most of the slides were stained with the Flemming's triple or with the fast-green triple combination (safranin, crystal violet, and fast green). Some smear preparations were also made of the lily anther according to the techniques of BELLING (2), MCCLINTOCK (16), and WARMKE (29).

Observations and discussion

The observations herein described extended over a period of many years and are reported for the first time. When the writer reported (21) the incomplete divisions of the sporogenous cells of the apogamous *N. hirtipes*, no reference was made to the cause of such divisions, although some evidence had already been obtained that in some way the tapetal cells might affect the behavior of the sporogenous cells.

Some evidence for the possible interaction of tapetal and sporogenous cells was later obtained from a study (25) of sporangia of *O. vulgatum* and *B. virginianum*. The observations of the tapetal phenomena in the eusporangiate ferns led to the discovery of incomplete divisions in their sporogenous cells. Since such divisions were found in both leptosporangiate and eusporangiate ferns, it was decided to study the sporangia of some vascular plants to determine, if possible, the cause of incomplete divisions in sporogenous cells which usually produce normal spores.

In the sporangia of *O. vulgatum* and *B. virginianum* it was observed that certain changes occur in some of the sporogenous cells, commonly in sporocytes during the first meiotic division. Cells which are undergoing the changes can be readily distinguished from their neighboring sporogenous cells. The former cells are usually confined to the periphery of the large group of sporocytes and hence are adjacent to the tapetum. The changes occur occasionally in solitary cells but more frequently in small groups of cells.

The most readily observed phenomenon in some of the sporocytes of the eusporangiate ferns is an incomplete division of their nuclei. This division occurs most frequently in prophase and less frequently in metaphase. Later stages in division in which some of the chromosomes have reached the poles of the spindle are found only occasionally. The restitution nuclei are of large size and usually nearly spherical in form. Some of these nuclei are, rarely, kidney or dumbbell shaped.

No chromosome counts were made in the restitution nuclei, but it appears certain, because of their large size, that they are polyploid. Whether the cells containing these nuclei actually function as spore mother cells was not determined.

In later stages of mitosis, usually beginning with metaphase, the cytoplasmic contents of the abnormal sporocytes become less dense. In some of the sporo-

cytes, undoubtedly tetraploid, a complete disintegration of the protoplasmic contents was observed to have occurred.

Since the phenomena that occur in the sporogenous cells of the eusporangiate ferns, as has already been stated, are accompanied by similar changes in the tapetal cells, it is suggested that in some way the action of the tapetal cells affects the behavior of the sporogenous cells.

Further evidence of this relationship has been obtained as a result of a study of these cells in two other plants—*N. hirtipes* and *L. longiflorum* var. *insulare*.

In the sporangium of the apogamous *N. hirtipes* the tapetum is composed of only two layers of cells, and the number of spores never exceeds sixty-four. In the large majority of the sporangia incomplete mitoses occur, and also incomplete cell divisions frequently occur during the eight-celled stage of the sporogenous cells. These cells usually function as spore mother cells and produce thirty-two normal haploid spores.

Hence, the sporangia of this fern are of two kinds: those in which there are produced sixteen haploid spore mother cells and sixty-four haploid spores and those in which there are produced eight diploid spore mother cells and thirty-two haploid spores. These observations confirm those of DÖPP (9).

Occasionally in some of the sporangia both haploid and diploid mother cells are formed which can readily be distinguished by a difference in size, the larger being diploid and the smaller haploid. Larger and smaller spores were sometimes found in the same sporangium. DÖPP (9) reported the smaller ones to be nonfunctional.

In numerous sporangia sporogenous cells, especially in the eight-celled stage, abort and hence produce no spores. Disintegration and final abortion of sporogenous cells have been observed by the writer in a number of other apogamous ferns.

The tapetum of the sporangia with sixteen spore mother cells is characterized by the usual nuclear and cell behavior. Hence, no unusual phenomena occur in sporangia which produce sixty-four spores. The tapetum of the sporangia in which diploid sporogenous cells are formed, however, is composed of larger cells, with dense cytoplasmic contents. Tapetal cells of this nature are also commonly present in sporangia in which the sporogenous cells abort.

As has already been stated, there are many sporangia in which the sporogenous cells undergo incomplete mitotic and cell division during the eight-celled stage. It is suggested that there may be an enzymatic action of the tapetal cells on the sporogenous cells which may be the cause of the interruption of the mitotic process and hence of the peculiar division. If the enzymatic effect on the sporogenous cells decreases after the disturbances in the divisions, the cells frequently form restitution nuclei and finally produce normal spore mother cells and spores. Since the tapetal cells increase in size and in density of their protoplasmic contents, it is suggested that they may obtain food from the sporogenous cells. The latter may, however, later digest the tapetum. If the postulated enzymatic action following the mitotic disturbance is increased, disintegration and final abortion of the sporogenous cells may result because of digestion by the tapetum.

Further evidence of an interaction between tapetal and sporogenous cells was obtained from a study of the microsporangia of a plant of *L. longiflorum* var. *insulare* which was found, upon investigation, to be anomalous in some respects.

Microsporangia of a normal plant were also studied to aid in the interpretation of the behavior in the abnormal one.

The tapetum in the sporangia of the normal plant consists of a single layer of cells (fig. 1). In the younger stages of the sporocytes the tapetal cells are usually uninucleate, and the nucleus divides frequently and in a normal manner. In the older tapetum the cells are commonly binucleate and often quadrinucleate. Although normal mitotic divisions occur, incomplete mitoses are not uncommon. When the sporocytes are about to enter synapsis, the large majority of the tapetal nuclei are spherical, but there are a few which are kidney or dumbbell shaped or quite irregular in form. At this stage nuclei of three sizes are found in the tapetum. The smaller ones are diploid with twenty-four chromosomes, the medium-sized ones are tetraploid, and the larger ones are octoploid with ninety-six chromosomes. Nuclei of three sizes were occasionally observed in a single cell. Chromosome counts were quite readily made in smear preparations.

Since the large majority of the polyploid nuclei in the tapetal cells of the normal *Lilium* are spherical in form, the incomplete mitoses must occur most commonly during prophase. Nuclei of other forms, already described, owe their origin to various irregularities in the mitotic process.

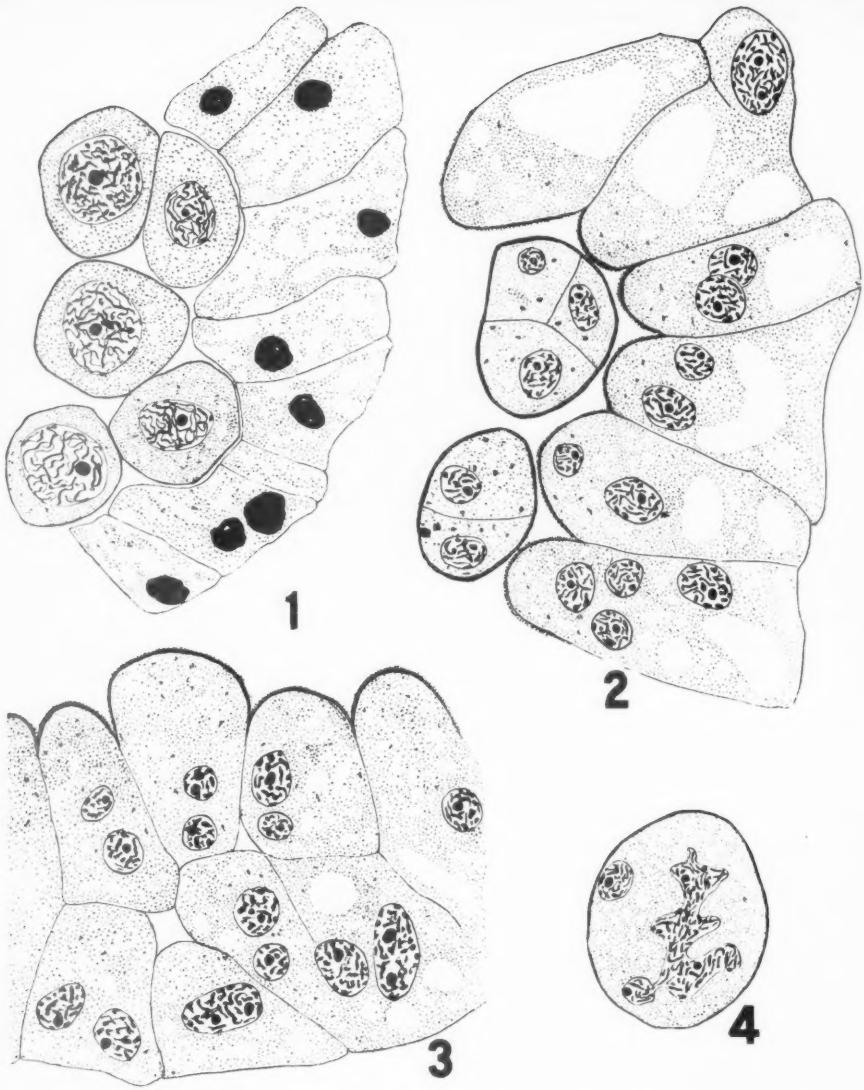
When anthers of the anomalous lily were examined, it could be observed that unusual changes had taken place in tapetal and sporogenous cells. In many microsporangia abortion of all or of some of the sporogenous cells had occurred. Therefore, at maturity these microsporangia were empty or contained only a few apparently normal spores. Frequently the tapetum had made a considerable amount of growth and almost filled the microsporangia by the time the nuclei of the spore mother cells were about to enter the meiotic prophase.

The tapetum often consisted of a single layer of cells (fig. 2) but occasionally of two layers (fig. 3). Hence, these tapetal cells, unlike those of the normal lily, retained the power to undergo cell division. The nuclei of these cells were usually of large size (fig. 3) and showed at this time no sign of disintegration. The cytoplasmic content of these cells was much denser than that of the corresponding cells of the normal plant. The inner walls of these tapetal cells were always considerably thickened (fig. 3), and it is suggested that such thickening only on the walls next to the sporogenous cells could be a result of material derived from the latter cells.

Occasionally, mitotic figures were observed in the tapetum during prophase stages in the spore mother cells. Incomplete mitotic and incomplete cell divisions were of less frequent occurrence than in the normal *Lilium*.

Incomplete mitoses, which can be seen so frequently in the sporogenous cells of *N. hirtipes* and in some other apogamous ferns, were found in only a few of the pollen sacs of the anomalous lily (fig. 4). Occasionally, large spores were observed. Since they were of such large size, they were, doubtless, polyploid.

The phenomena which have been described in the anther sacs of the anomalous *Lilium* may be stated as follows: (*a*) an increased activity in the tapetum which may become two cell layers thick; (*b*) prolonged activity of the tapetal cells; (*c*) an increase in the size of the tapetal cells and in the density of their cytoplasmic contents; (*d*) an increase in the thickness of the inner walls of these cells; (*e*) fewer incomplete mitoses in the tapetal cells and consequently a decrease



FIGS. 1-4.—*Lilium longiflorum* var. *insulare*, tapetal and sporogenous cells. × 420. Fig. 1, portion of tapetum and sporogenous cells from sporangium of normal plant. Tapetum consisting of single layer of cells is disintegrating; sporogenous cells in meiotic prophase. Fig. 2, portion of tapetum from median section of microsporangium of anomalous lily. Tapetum consists of single layer of cells with dense cytoplasmic contents. A few apparently normal tetrads were produced in sporangium—two are shown. Fig. 3, as in fig. 2. Many tapetal cells are binucleate and are characterized by density of cytoplasmic contents. Inner walls of cells are considerably thickened. Sporogenous cells in sporangium were in meiotic prophase. Fig. 4, large spore mother cell of anomalous lily showing irregularity in nuclear division.

in the number of polyploid nuclei; (*f*) incomplete mitoses of the spore mother cells, which may occasionally produce polyploid spores; and (*g*) partial or complete abortion and disintegration of the spore mother cells.

From the foregoing observations of tapetal and sporogenous cells of the anomalous lily and of the eusporangiate and leptosporangiate ferns, it is suggested that there may be an interaction between these two kinds of cells.

It is a well-established fact that under ordinary conditions the sporogenous cells in the sporangia of vascular plants may, by means of enzymes they secrete, digest the tapetal cells. It is here suggested that under certain other conditions the tapetal cells may secrete enzymes which can produce changes in the sporogenous cells similar to those in the ordinary tapetal cells of vascular plants.

Summary

1. An interaction between tapetal and sporogenous cells in vascular plants is suggested.

2. Tapetal phenomena in normal sporangia are well known to be the following: (*a*) incomplete nuclear and cell division; (*b*) formation of polyploid nuclei; (*c*) formation of binucleate and quadrinucleate cells; (*d*) decrease in the cytoplasmic contents of the cells; and (*e*) digestion of tapetal cells as a result of enzymatic activity of sporogenous cells.

3. The above phenomena also occur under certain conditions in the sporogenous cells. The behavior of these cells, it is suggested, may result from the activity of tapetal enzymes. The changes which occur in the tapetum under these conditions are as follows: (*a*) an increase in the mitotic activity and in the period of time during which cell division may occur; (*b*) formation of two layers of cells; (*c*) an increase in the size of cells and in the density of their cytoplasmic contents; and (*d*) a decrease in the number of incomplete mitoses, and hence a decrease in the number of polyploid nuclei.

4. The sporogenous cells are affected in two ways: (*a*) they may be partially or completely digested, and hence, at maturity, the sporangium may contain only a few spores or no spores at all, and (*b*) interruptions of the mitotic activity resulting from the activity of an enzyme or enzymes produced by the tapetal cells may lead to incomplete mitotic division and a consequent doubling of the chromosome number. These cells may undergo meiosis and produce normal spores, as in some of the apogamous ferns.

5. It is impossible at present to state with certainty the conditions under which the behavior of the sporogenous cells simulates that of the tapetal cells. Experimental evidence may, perhaps, be necessary to account for the behavior of the cells in the sporangia of certain vascular plants.

6. If there is an interaction between tapetal and sporogenous cells, as is suggested, it is possible to account partially for the sterility reported in some pteridophytes, especially some of the ferns, and in a number of seed plants.

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IN VITRO CULTURE OF THE COTTON EMBRYO[1]

HUGH B. LOFLAND, JR.

Introduction

Various authors have reported that the immature embryos of a variety of plants are able to grow and develop *in vitro*. Numerous workers report results which range from complete failure of the embryo to develop to growth in which a normal seedling is produced. In general, the results have depended on the species of plant, the culture medium used, and the age at which the embryo was excised from the seed.

As early as 1904 HANNIG (2) reported the growth *in vitro* of the embryos of *Raphanus* and *Cochlearia* after they had been removed from their ovules. STINGL (7), in 1927, succeeded in culturing the embryos of several grains. More recently, a large number of articles have appeared describing the growth of the embryos of various plants. In 1932 WHITE (13) obtained good growth of *Portulaca oleracea*. TUKEY (8, 9, 10), LA RUE (4, 5), VAN OVERBEEK (11, 12), and KENT and BRINK (3) have grown embryos taken in different stages of development from a wide variety of plants.

The work reported in this paper was an investigation of the growth characteristics of the immature embryos of American Upland cotton, *Gossypium hirsutum*. It would be of advantage to be able to grow to maturity the embryos resulting from certain "nonviable" crosses of different strains of cotton. To the author's knowledge there are no reports in the literature of the successful culture of cotton embryos. For this reason the work was carried out to determine (*a*) if the immature embryos of cotton can be induced to grow *in vitro* and (*b*) to study the characteristics of the growth resulting from such culture. Normal embryos, rather than those from faulty or abortive bolls, have been used, so that basic information may be gained on the development *in vitro* of normal embryos. If a satisfactory technique can be found for culturing these embryos, a great deal of information can be derived by applying these techniques to "nonviable" or "physiologically sterile" seeds.

Material and methods

Both field-grown and greenhouse plants were used. The ages of the embryos were determined by tagging flowers at anthesis (day of fertilization). The embryos were removed from the tagged bolls and placed on media as soon as possible after removal of the bolls from the plant, usually within 1–2 hours. The technique for removal of the embryos from the seeds was as follows: Tagged bolls were opened, and the seeds removed immediately and placed in a sterile Petri dish. The seed coat was sterilized by dipping the whole seed in alcohol and flaming. The seed was then opened, using a sterile scalpel and forceps, and the embryo removed and placed on the surface of the sterile medium contained in a 22 × 80-mm. glass screw-top vial. By use of ordinary precautions of aseptic technique, very little contamination resulted from handling the embryos in this way.

The media consisted of the routine medium recommended by WHITE (14)

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for the culture of plant tissues (table 1). Various supplements were added as desired. Agar was added to the basal solution in a concentration of 0.75%, and the pH value was adjusted to about 5.8. All media were sterilized by autoclaving, except in those cases where the media contained thermolabile substances or such plant extractives as malt extract, cotton extract, or other substances which deteriorate on heating. Media containing these substances were sterilized by filtration through a Seitz filter.

TABLE 1

COMPOSITION OF BASAL MEDIUM

Substance	Concentration (mg/liter)	Substance	Concentration (mg/liter)
$MgSO_4$	360.0	$ZnSO_4$	1.5
$Ca(NO_3)_2$	200.0	H_3BO_3	1.5
Na_2SO_4	200.0	KI	0.75
KNO_3	80.0	Sucrose	2000.0
KCL	65.0	Glycine	3.0
$NaH_2PO_4 \cdot H_2O$	16.5	Nicotinic acid	0.5
$Fe_2(SO_4)_3$	2.5	Pyridoxine	0.1
$MnSO_4$	4.5	Thiamine	0.1

Except where specifically mentioned, the cultures were incubated at room temperature in the ordinary light of the laboratory.

DEVELOPMENT OF NORMAL EMBRYO.—REEVES and BEASLEY (6) traced the development of the cotton embryo both morphologically and chemically. They found that at 9 days after fertilization the main organs—cotyledons, hypocotyl, and plumule—were formed and that the embryo then entered on the period of rapid growth. At 9 days it is less than 1.0 mm. long; at 15 days approximately 4 mm.; at 20 days 7.7 mm.; and about the twenty-sixth day after fertilization it reaches a maximum length of about 10 mm. Maximum weight is reached about the thirty-fourth to thirty-fifth day.

ALTSCHUL (1) stated that cottonseeds from freshly opened bolls do not usually germinate so promptly as do normal dry seeds. Therefore, the embryo at 25–35 days, although having reached maximum size, is still immature when judged by its ability to germinate in soil. It will later be shown that these embryos will grow *in vitro*, however, and develop to produce a normal seedling.

The attempts to culture these embryos have been carried out principally at three main levels of embryo development. These stages are: (*a*) the 27-day-old level, at which time the embryos are mature in size and develop normally on most media; (*b*) the 20–21-day level, at which time the embryos are immature, measuring 7–8 mm. in length, and the cotyledons are just beginning to curl upward around the radicle; and (*c*) the 15–16-day level, when the embryos measure 3–4 mm. in length and are flat. Embryos 27 and 20 days old are shown in figure 1*A*, *B*.

Experiments were devised in which attempts were made to culture embryos of the three age groups mentioned above, using a variety of media and environmental conditions. The experiments were planned from the practical point of view of finding a medium and/or condition which would support rapid development of immature embryos.

Results and discussion

In those experiments in which the pH value of the medium was varied between 5.0 and 8.0, the growth response was about the same over the entire series. Incubation temperatures ranging from 20° to 30° C. similarly showed no effect. When the amount of light was varied, ranging from incubation in the dark to 3–4 hours per day illumination with a 50-watt bulb, the only observable effect

was in the rapidity with which the cotyledons developed chlorophyll. Embryos kept in the light developed chlorophyll in the cotyledons rapidly, even in the immature stages.

Among the supplements which have been added to the basal medium are: (*a*) complete B-vitamin solution, (*b*) a fatty acid solution in the form of the water-soluble "Tween 40," (*c*) a complete amino acid mixture, and (*d*) the following carbohydrates, added individually to the basal medium: sucrose, lactose, glucose, starch, galactose, arabinose, levulose, raffinose, and xylose. In all cases it was observed that supplementing the basal medium with these substances resulted in no better growth than that of the control embryos planted on the basal medium without supplement.

Numerous workers have found growth-stimulating factors to be present in a variety of naturally occurring substances. VAN OVERBEEK (11) stated that a medium containing coconut milk gave a five-hundred-fold increase in the size of *Datura* embryos in 6 days. In another paper (12) he reported "embryo factor" activity in yeast extract, wheat germ, and almond meal. KENT and BRINK (3) discovered growth-stimulating properties for *Hordeum* embryos in tomato extract, casein hydrolysate, and sodium nucleate. They found that, when casein hydrolysate was used, the embryos cultured *in vitro* sometimes grew to a size exceeding that of the embryos left on the plant.

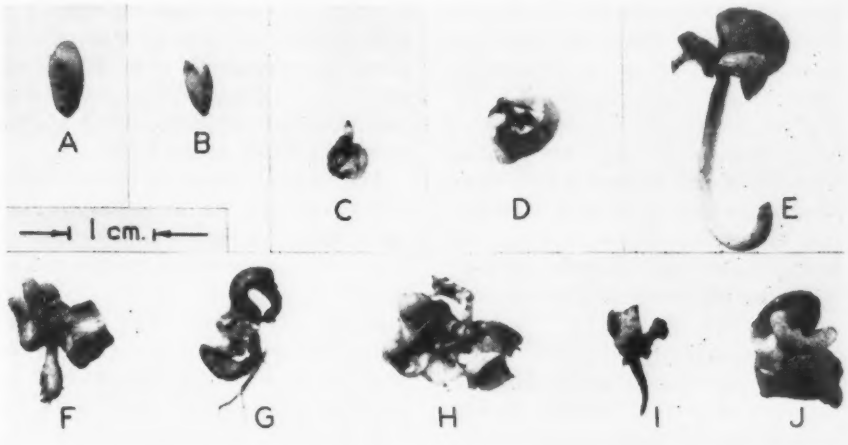

FIG. 1.—*A*, *B*, normal embryos removed from seed at 27 and 20 days after fertilization. *C*, embryo excised at 15 days; alive and growing at 3 weeks. *D*, embryo excised at 20 days, growing on basal medium at 3 weeks. *E*, seedling at 7 days grown from embryo excised when 27 days old. *F*, *G*, *H*, *I*, *J*, abnormal forms showing lateral root formation and twisted shapes at 3 weeks.

The results of the author's investigations in applying such growth-stimulating substances to cotton embryos have not been successful in bringing about more rapid growth of the embryos. Tomato extract, coconut milk, casein hydrolysate, sodium nucleate, and an extract made from the ovules of developing cotton bolls have all been used. The results have nearly all been uniformly neg-

ative, in that the substances do not alter the growth rate of the cotton embryos. An exception may be made in the case of casein hydrolysate, since preliminary results seem to indicate that a higher percentage of embryos grow in the presence of low concentrations of this substance; at present, however, the data are not conclusive.

All the foregoing data have indicated negative results in the search for a substance or a set of conditions which would promote the rapid growth of excised cotton embryos. It should be emphasized, however, that these embryos can be cultured *in vitro* on the basal medium of WHITE. Supplementing this medium with 0.5% of malt extract or with casein hydrolysate seems to favor this development. The growth is slow, however, and frequently abnormal. A summary of the growth characteristics of these embryos is given below.

Embryos excised and placed on the basal medium when 27 days old usually show signs of activity within 2–3 days after planting. Elongation of the radicle occurs, the cotyledons unfold, and chlorophyll appears in the cotyledons. Further growth occurs to produce a normal seedling. The seedling is usually somewhat smaller than that produced by a dry mature seed. Figure 1*E* shows in an early stage of development a seedling grown from an embryo excised at 27 days. Abnormalities in growth are frequently noticed, however, even at this advanced stage. The radicle often fails to develop, and lateral roots are produced from its tip. Some embryos become gnarled and twisted (fig. 1*F–J*).

When excised at the 20-day level of growth, fewer of the embryos develop than when removed at 27 days; growth is also slower, and a higher percentage of abnormal forms are produced. Figure 1*D* shows an embryo excised when 20 days old that has been growing for 60 days on the basal medium containing casein hydrolysate. When embryos of this age do produce seedlings, they are small and spindly and usually do not survive to reach maturity.

Figure 1*C* shows an embryo excised at 15 days which has been on basal medium for 60 days. Chlorophyll has developed in the cotyledons, but the rate of growth is extremely slow. One such embryo, however, has grown to produce a small plant, complete with roots and hypocotyl. Its total length was 50 mm. As a general rule, embryos excised at 15 days grow very slowly or not at all.

The over-all picture of the culture of cotton embryos as indicated by this study seems to suggest that cotton follows the same pattern of growth *in vitro* as that described for certain deciduous fruits by TUKEY (10), who found that embryos enter at once, when removed from the seed, into an independent development characteristic of the age of the embryo when excised and do not pass through the embryonic stages characteristic of embryos on the mother plant. This would seem to be indicated in cotton by the fact that in no case have we been able to get a continuation of an embryonic type of growth. Instead, the embryo develops chlorophyll and grows to produce a miniature seedling much smaller than one produced from a normal, mature seed.

It may be concluded that additional work should be done to discover a medium, growth factor, or set of conditions which will stimulate (*a*) more rapid development of excised embryos and (*b*) a continuation of the embryonic type of growth which would allow the embryo to develop to a size approximately equal to that which it would attain if left on the

plant. This would result in the production of hardier seedlings and enhance the probability that the plant might grow to maturity.

Summary

1. The techniques and methods used in the study of the growth *in vitro* of cotton embryos have been described. Embryos were excised from the seed at 27-, 20-, and 15-day-old stages and planted on the basal medium of WHITE, supplemented with vitamins, a fatty acid solution, amino acids, and various carbohydrates. In addition, coconut milk, tomato-juice extract, sodium nucleate, and casein hydrolysate have been used as supplements to the basal medium. In general, these substances are without effect in promoting rapid growth of the embryos.

2. Embryos excised at 27 days of age usually grow rapidly when placed on the basal medium, and normal seedlings are produced. Embryos excised at 20 days grow very slowly, and many abnormal forms are produced. Embryos excised at 15 days usually fail to grow; when growth does occur, it is slow, and a very small, weak seedling is produced.

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NATURALLY OCCURRING POLYPLOIDY IN THE DEVELOPMENT OF ALBIZZIA JULIBRISSIN DURAZZ.[1]

C. A. BERGER AND E. R. WITKUS

Introduction

Certain species of diploid plants contain some polyploid cells which are formed as a part of the normal process of development. In some forms, as in *Spinacia oleracea* L. (1), this formation of polyploid cells continues throughout the life of the plant. In other species the formation of polyploid cells is restricted to a definite period in the developmental process. Two cases of the latter type have recently been reported by the present investigators (2, 3). In *Allium cepa* the polyploid cells were found in the large cortical cells of the shoot of seedlings of from 10 to 40 mm. in length. In *Mimosa pudica* a similar phenomenon was found in the roots of seedlings of from 2 to 10 mm. in length. In both cases only tetraploid cells were found, and these cells underwent only one division in the tetraploid condition. Another recently studied case of the same phenomenon, found in the silk tree, *Albizzia julibrissin* Durazz., contains some interesting variations and is here reported.

Observations

The diploid chromosome number of *Albizzia julibrissin* is twenty-six. Polyploid mitoses were found in root tips of young seedlings of *Albizzia* ranging from 2 to 30 mm. in length. About two dozen polyploid metaphases were counted in each 2-mm. root. The frequency of such metaphases increased to a maximum of about two hundred in 5-mm. roots and then decreased to about twenty-four in 20-mm. seedlings. Only occasionally was a polyploid division observed in roots of seedlings 30 mm. in length, and no such divisions were found in root tips of mature plants. The meristematic region of root tips of mature plants is about one-third the length of the similar region in root tips of seedlings. Since only metaphase stages were counted, the number of polyploid divisions and of polyploid cells was much higher than the figures given. With the exception of one octoploid cell, the degree of polyploidy found was always the tetraploid.

Diploid and tetraploid metaphases are shown in figures 1–3. The tetraploid divisions were always found in large cells located in the periblem, in the dermatogen, and, rarely, in the large central cells of the plerome. This distribution of the polyploid cells through all three regions of the primary meristem is in sharp contrast to the situation in *Spinacia*, where they are restricted to the periblem. A similar difference between the two species is found in the delimitation of the three primary meristematic regions in the two species. In *Spinacia* the dermatogen, periblem, and plerome are very sharply set off from one another, while in *Albizzia* there is no sharp differentiation between the three regions, so that it is frequently difficult to determine whether a particular cell belongs to the dermatogen or to the periblem. This difference between the two species in the distribution of polyploid cells and in the differentiation of the primary meristematic regions may be causally correlated.

In *Allium* and in *Mimosa* all the poly-

[1] Aided by a grant from the National Advisory Cancer Council.

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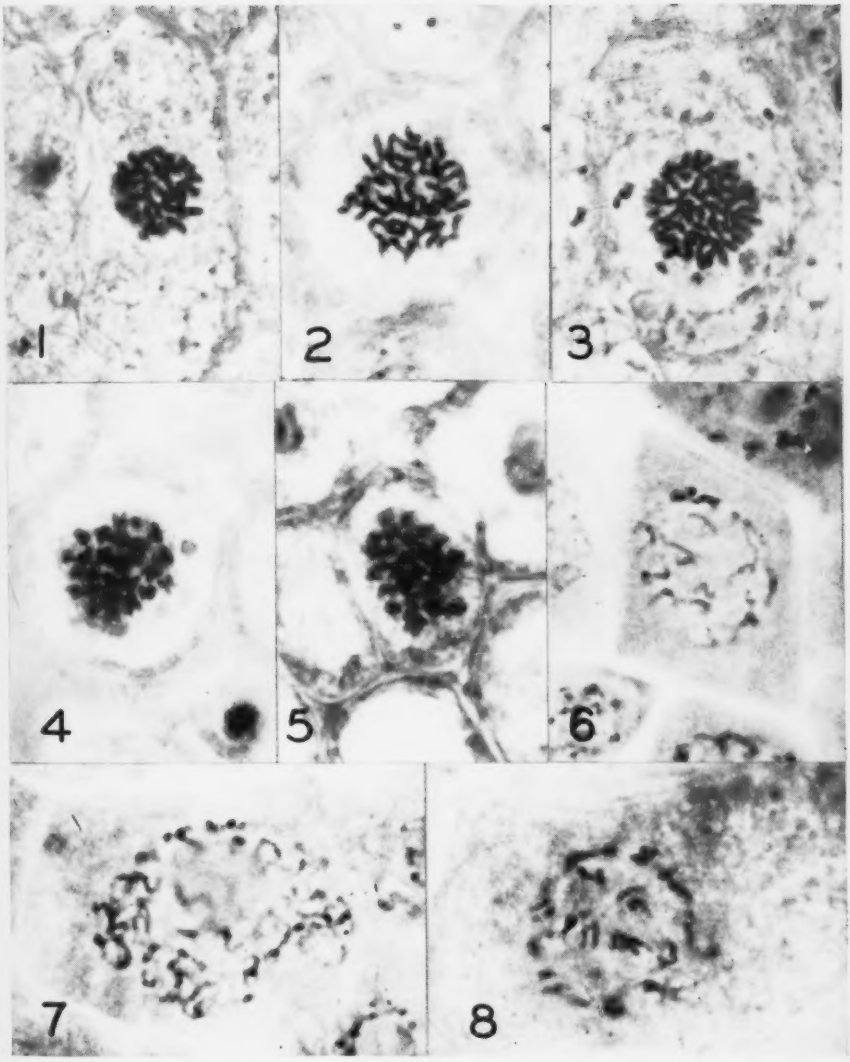

FIGS. 1–8.—Photomicrographs of aceto-orcein smear preparations of root tips of *Albizzia julibrissin*. ×1750. Fig. 1, diploid metaphase. Fig. 2, paired tetraploid metaphase. Fig. 3, unpaired tetraploid metaphase. Fig. 4, paired tetraploid metaphase with supercontracted chromosomes. Fig. 5, tetraploid metaphase with closely paired supercontracted chromosomes. Fig. 6, diploid prophase. Fig. 7, tetraploid prophase with unpaired chromosomes. Fig. 8, tetraploid prophase with paired chromosomes.

ploid divisions showed closely paired chromosomes, indicating that the cells underwent only one division as polyploids. In *Albizzia* both paired and unpaired metaphases were found (figs. 2, 3) in dividing polyploid cells, showing that at least some of these cells underwent more than one division as polyploids before becoming differentiated. The paired and unpaired types of polyploid divisions are clearly distinguishable in prophase. Figure 6 shows a diploid prophase with unpaired chromosomes, figure 7 a tetraploid prophase with unpaired chromosomes, while figure 8 shows a tetraploid prophase with paired chromosomes.

Another interesting variation found in *Albizzia* was the presence of two kinds of paired tetraploid metaphases. One type, as shown in figure 2, has moderately contracted chromosomes which are loosely paired while the other and rarer type (figs. 4, 5) has very closely paired chromosomes which are supercontracted. No clue as to the significance of these two types of paired metaphases was found. Since paired polyploid metaphases are found among the earliest divisions in very young seedlings, it is probable that the formation of polyploid cells begins during the development of the embryo.

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INFLUENCE OF GROWTH "HORMONES" ON BOLL RETENTION BY COTTON PLANTS[1]

FRANK M. EATON[2]

Introduction

In view of the notable effects of growth substances on plant development it is not surprising that questions should be recurrently raised about the possibility of increasing boll retention by cotton plants through the use of the various synthetic materials. Used as a spray, naphthaleneacetic acid delays the abscission of mature apples, but this substance is also an effective thinning agent for the young fruit of both apples and peaches; thus, according to the stage of fruit development, the same material may either retard or cause abscission.

It is well known that the cotton plant, during the fore part of its fruiting period, tends, in the absence of insect or other injury, to retain almost all its young bolls. But, as the summer progresses and the boll load carried by the plant increases, successively greater percentages of the new bolls are shed within a few days after anthesis. The cotton plant,

[1] Contribution from the Division of Cotton and Other Fiber Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U.S. Department of Agriculture, and Texas Agricultural Experiment Station, College Station, Texas.

[2] Principal Plant Physiologist.

under suitable conditions, can be exceedingly efficient in the proportion of its dry weight laid down in the form of seed cotton; in productive strains the seed cotton commonly represents 60%, or more, of the final dry weight of the above-ground portion of the plant. There must, accordingly, be some limit to how much further such reproductive efficiency could be extended. If, for example, a means were found for causing a greater percentage of the early flowers to develop into bolls, then it might only follow that those developed would be smaller. In fact, small-bolled cottons customarily mature more bolls and shed fewer bolls than the large-bolled varieties. Additional early bolls, if a means were found for causing them to set, would add to the determinateness of the cotton plant and thereby tend to limit its further development and flowering. Occasionally, here and there, a little extra early-season shedding increases, rather than decreases, the ultimate yield.

On the other hand, cotton plants are not always fruited in proportion to their vegetative development, and in such cases a means of increasing floral-bud and boll retention could add greatly to yields. Low yields have been noted as a heritable characteristic in hybrids (3). Some good strains occasionally fruit poorly without any unusual prevalence of insects now recognized as being injurious. Cotton strains that are not very determinate shed more of their early bolls than do determinate strains; the cause of this extra shedding is unknown. According to circumstances there thus appear to be reasons both for and against a conclusion that the use of synthetic substances with hormone-like activities might alter the course of shedding and retention of cotton buds and bolls. But it does not follow, necessarily, that the growth substances now known or commercially available could be used to increase the retention of floral buds or contribute to the retention and development of bolls after anthesis.

In all probability, various experiments to determine the effects of synthetic growth materials on boll shedding have been conducted by others, even though the results have not appeared in the literature. In 1945 one of the agricultural-chemical manufacturers made available and sent out for trial use a material "for aiding in cotton boll setting."

Material and methods

In 1947 and again in 1948 dusts of sodium 4-chlorophenoxyacetate and sodium alpha-naphthaleneacetate were used on cotton plants during August at a time when substantial percentages of the newly formed bolls were shedding. At such a time the shedding tendency can change in either direction, and the effects of the substances on trial, favorable or unfavorable, should therefore be most evident. In 1948 cotton plants were sprayed weekly, starting in June, with 10- and 20-p.p.m. solutions of the sodium salt of beta-naphthoxyacetic acid, in addition to sprays of the two other chemicals mentioned above. These experiments were conducted at the United States Cotton Field Station, Shafter, California. Also, both at Shafter in the summer and at College Station, Texas, in the winter, short-day cotton varieties have been sprayed with various growth substances to determine if the type of branches could be altered by this means.

Experimentation

LATE SEASON DUSTING.—The dusting experiments reported in tables 1 and 2 gave similar results in that in no instance did the materials used have statistically significant effects on the percentages of

bolls retained. In each experiment the eight to ten plants in each plot were covered with cotton sheeting while being dusted so that heavy applications could be made without danger of a drifting of the dusts to neighboring plants. The 1948 experiment (table 2) included three varieties of cotton, each with a different planting date and each in a different part of the station. With the different locations there were associated some differences in soil, irrigation dates, plant maturity, etc. In each soil there was a large supply of available nitrogen.

TABLE 1

PERCENTAGE OF BOLLS RETAINED BY ACALA × MISDEL COTTON PLANTS IN THREE EXPERIMENTS AT SHAFTER, CALIFORNIA, 1947

A. *Sodium 4-chlorophenoxyacetate as 100-p.p.m. dust applied on August 5. Total of 543 flowers were tagged during 6-day period starting August 4*

Treatment	Block				Means
	1	2	3	4	
Control	31.8	28.1	23.9	19.4	25.8
Dusted	34.6	32.3	25.0	19.4	27.8

B. *Naphthaleneacetic acid as 1000-p.p.m. dust applied in blocks 1 and 2, and as 25-p.p.m. spray in blocks 3 and 4. Total of 283 flowers were tagged during 6-day period starting August 4*

Treatment	Block				Means
	1	2	3	4	
Control	28.3	33.3	23.7	42.3	31.9
Dusted	23.1	22.5	20.5	19.5	21.4

C. *Sodium 4-chlorophenoxyacetate (100 p.p.m.) and naphthaleneacetic acid (1000 p.p.m.) dusts applied on August 8. Total of 337 flowers were tagged during 6-day period starting August 7*

Treatment	Block				Means
	1	2	3	4	
Control	10.3	23.3	10.0	35.9	20.6
Dusted	17.9	18.8	29.7	32.4	27.3

WEEKLY SPRAYS.—The spraying experiment reported in table 3 was undertaken to determine not only possible effects of growth "hormones" on the fruitfulness of the cotton plant but also their effects on its vegetative development. The sodium salts of 4-chlorophenoxyacetic acid, beta-naphthoxyacetic acid, and alpha-naphthaleneacetic acid were used in concentrations of 10 and 20 p.p.m., each chemical and concentration being replicated three times in randomized plots of ten plants each. The variability of plant size in the area indicated that it might be unwise to randomize the two concentrations of the three materials in a single test at the cost of greater separation between the controls and the various treatments. The three replicates of the 10-p.p.m. experiment were, therefore, randomized in segments of three adjacent rows of cotton. An additional three blocks were staked out similarly in the next three rows for a duplication of the treatments but with 20-p.p.m. sprays. The spraying was started on June 19, which was about 2 weeks in advance of active flowering, and continued until late August. A spreader was mixed with the spray, and the plants were well wetted at each operation. Plant measurements were made on August 24 and 25.

Sodium 4-chlorophenoxyacetate was the only one of the three compounds that induced symptoms. When applied weekly in a concentration of 20 p.p.m., a notable flaring of the margins of the younger leaves resulted, about half the leaves of these plants being affected in some degree. At 10 p.p.m. only the youngest leaf or two on each branch was affected. When leaf margins were flared, there was always a tendency for the leaf veins to be prominent.

TABLE 2

PERCENTAGES OF BOLLS RETAINED FROM FLOWERS APPEARING DURING TWO PERIODS (AUGUST 15–19 AND 20–24) BY THREE VARIETIES OF COTTON AT SHAFTER, CALIFORNIA, IN 1948. DUSTED ON AUGUST 15 WITH SODIUM 4-CHLOROPHENOXYACETATE APPLIED AT CONCENTRATION OF 100 P.P.M. AND NAPHTHALENEACETIC ACID AT 1000 P.P.M

Treatment	Variety and block						Total flowers	Bolls retained	
	Acala 4-42		Acala p 18-C		Acala×Misdel			By periods	Total*
	1	2	3	4	5	6			
A. *Sodium 4-chlorophenoxyacetate:*									
Period 1	42	66	30	57	13	51	365	40.4	37.6
Period 2	73	17	27	24	11	45		34.5	
B. *Naphthaleneacetic acid:*									
Period 1	33	32	35	43	35	45	343	37.6	38.3
Period 2	40	70	42	28	21	46		39.1	
C. *Equal parts A and B:*									
Period 1	44	38	38	28	47	33	353	38.1	35.9
Period 2	38	100	31	6	36	48		33.5	
D. *Control:*									
Period 1	46	38	47	33	35	64	351	36.0	36.2
Period 2	67	91	36	48	24	50		36.3	

* Calculated from total flowers produced and bolls retained.

TABLE 3

GROWTH AND FRUITING OF PLANTS SPRAYED WEEKLY THROUGHOUT SUMMER, 1948, WITH 20 P.P.M. CONCENTRATIONS OF SODIUM SALTS OF THREE GROWTH SUBSTANCES

Plant characters measured	Control	Treatments			Difference required for significance at 5% level
		4-chlorophenoxyacetate	Beta-naphthoxyacetate	Naphthaleneacetate	
Bolls per plant, no.	24.6	20.3*	29.0*	27.0	3.7
Fresh stems and leaves, gm.	415	535*[1]	450	425	106
Bolls per 100 gm. fresh stems and leaves, no.	6.1	3.9*	6.4	6.1	1.2
Height, cm.	104	114*	108	103	9.0
Main-stalk nodes, no.	25.3	28.3*	26.6	26.0	1.6
Longest fruiting branch, cm.	41	46	44	45	7
Longest fruiting branch nodes, no.	4.5	4.8	4.7	4.8	2
Vegetative branches over 20 cm., no.	1.7	2.2*[1]	1.6	1.7	0.9
Vegetative branches over 20 cm., length, cm.	97	136	90	86	56

[1] In these two analyses of variance "treatment" as a whole lacked significance, but by individual comparisons the weight of fresh stems and leaves was highly significant, and the number of vegetative branches was significant.

The plant characters selected for the measurement of the responses (table 3) provide effective descriptions of the influence of growth conditions on the development of the cotton plant. The number of bolls per 100 gm. of fresh leaves and stems (relative fruitfulness) is regarded by the writer as being a more critical measurement of the tendency of the cotton plant toward reproductive or vegetative growth, respectively, than is the more commonly used percentage of flowers or bolls shed. In the event of any variability in floral-bed shedding the latter measurement, taken by itself, becomes almost meaningless when used over a season's growth. Inasmuch as it has been found that the growth rate of a cotton boll is almost linear from a few days after flowering until a few days before opening, the number of developing bolls per 100 gm. of leaves and stems provides, within a variety, a creditable index of the carbohydrate disposition between vegetative and reproductive activities. Since this index is based on plant size, it effectively indicates whether the fruiting efficiency of the plant has been high or low.

The measurements from the spray experiments showed little evidence approaching statistical significance of any effects of the 10-p.p.m. concentrations of any of the three materials. The data of table 3 are accordingly confined to the effects of the 20-p.p.m. sprays. Among the 20-p.p.m. treatments the beta-naphthoxyacetate and naphthaleneacetate likewise, with one exception, failed to have significant effects on the plant characters measured. On the other hand, some rather notable effects resulted from the use of sodium 4-chlorophenoxyacetate. Significant reductions are found in the number of bolls per plant and also in the number of bolls per 100 gm. of fresh stems and leaves. Coupled with these effects there were significant gains in the fresh weight of the stems and leaves, in the number of main-stalk nodes, and in the number of vegetative branches. Other gains that lack statistical significance were found in the length and number of nodes of the fruiting branches and in the average length of vegetative branches. The 10-p.p.m. concentration, but to a lesser extent than the 20-p.p.m. treatment, reduced the relative fruitfulness of the plants and brought about an increase in the number and length of the vegetative branches and the length of the fruiting branches; but it did not induce increases in plant weight, height, or number of fruiting-branch nodes.

With the foregoing effects considered collectively, sodium 4-chlorophenoxyacetate in concentrations high enough to induce symptoms resulted in reduced fruiting and in increased vegetative development. A similar effect on vegetative growth has been repeatedly observed to result from the mechanical removal of bolls and from insect damage that causes shedding. In the present experiments it is, to the writer, most reasonable to believe that sodium 4-chlorophenoxyacetate caused bud or flower shedding and that the increased vegetative development of the plant was a direct consequence thereof. On the other hand, some investigators think of cotton plants that are in a highly vegetative condition as being unable to fruit heavily. In this latter light, it is possible that the poor fruiting of the plants sprayed with sodium 4-chlorophenoxyacetate was a result of stimulated vegetative activity rather than the converse. The interpretation based on increased abscission would be in keeping with the observations of MURNEEK and HIBBARD (4) on the effect of spring applications of "hormone" sprays to peach

trees and with those of L. P. BATJER (unpublished) to apple trees. The cotton plants treated with 20 p.p.m. of sodium 4-chlorophenoxyacetate carried only 64% as many bolls per unit of fresh stems and leaves as did the control plants.

EFFECTS ON BRANCH TYPE.—Many observations suggest reasons for likening, or regarding as homologous, the fruiting branches of the cotton plant to the inflorescences of other plants. Under certain conditions the cotton plant can develop either vegetative or fruiting branches to the exclusion of the other type. Usually, however, two or three vegetative branches are initiated from nodes near the base of the main stalk during early growth. Planting rates and nitrogen supply, as well as quite a number of other factors (2), can alter this behavior. During long days, short-day cottons produce only vegetative branches. Cold nights can cause day-length neutral strains to produce only vegetative branches (1). Fruiting branches may arise from the nodes of vegetative branches as well as from the main stalk. Vegetative branches may likewise arise from fruiting branches as well as from the main stalk. Only fruiting branches produce flowers.

To learn if the branching behavior of cotton might be altered by the use of any of the foregoing growth substances, all were used as sprays in one or more experiments in the summer and in the winter on both day-length neutral as well as short-day strains of cotton. In no instance were the proportions of branches characteristic of the cotton plants under the existing conditions materially altered. It was noted that some of the growth substances when used in concentrations sufficient to depress all branch development still permitted a little growth and leaf development by the terminal bud.

Summary

1. Under climatic conditions of the San Joaquin Valley of California dusting cotton plants during periods of active shedding with 100 p.p.m. of sodium 4-chlorophenoxyacetate and 1000 p.p.m. of naphthaleneacetic acid was without effect on boll retention in both 1947 and 1948.

2. Spraying plants weekly with a 20-p.p.m. solution of sodium 4-chlorophenoxyacetate, in an experiment where naphthaleneacetate and napthoxyacetate were without effect, significantly reduced the number of bolls per plant and the number of bolls per 100 gm. of stems and leaves and caused some increase in vegetative development.

3. The fruiting branches of the cotton plant are in some respects homologous to the inflorescences of other plants—that is, under certain conditions cotton can develop either vegetative or fruiting branches to the exclusion of the other type. Only the fruiting branches produce flowers. Various synthetic-hormone sprays used on various types of cotton and under various conditions did not materially alter the kind or proportions of branches developed.

4. The foregoing data provide little basis for believing that the late-season setting of cotton bolls can be increased by the use of the type of growth-regulating materials employed or for believing that these materials can alter materially the proportions of fruiting and vegetative branches. A florigen type of growth substance might, however, have marked effects. Also, the anti-auxin type of material remains to be studied.

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EFFECTS OF 2,4-D AND OTHER GROWTH SUBSTANCES ON PHOTOSYNTHESIS AND RESPIRATION IN ANACHARIS

R. O. FREELAND

Introduction

Because of the many and varied practical applications that have been discovered for 2,4-dichlorophenoxyacetic acid (2,4-D) and other growth substances, many investigators are interested in trying to find the relationships which may exist among the application of these compounds to plants, the physiological processes involved, and such resulting phenomena as rooting, gall formation, fruit set, and death. In this connection much has been published regarding the effects of 2,4-D and other growth substances on respiration. Recent papers by KELLY and AVERY (2) and MITCHELL *et al.* (5) contain additional data and brief reviews of the literature on this subject. Apparently the various substances which have been tested may retard, accelerate, or have no measurable effect upon respiration, depending upon what the growth substance is, its concentration, and the kind of plant.

On the other hand, very few data are available in the literature regarding the effects, if any, which specific auxins and growth substances have upon the process of photosynthesis. MITCHELL *et al.* (4) found that bean plants which had been depleted of carbohydrates by being kept in the dark accumulated less sugar, starch, and dextrin during subsequent illumination when they were treated with alpha-naphthalene acetic acid than similar untreated control plants. They suggested that a reduction in the rate of photosynthesis would provide a possible explanation of the data. FREELAND (1) reported the effects of 2,4-D, indole-3-acetic, gamma-(indole-3)-*n*-butyric, beta-naphthoxyacetic and para-chlorophenoxyacetic acids on photosynthesis in bean plants. He concluded that the use of each of these substances, except naphthoxyacetic acid, resulted in a decrease in apparent photosynthesis when applied as a mist spray at a concentration of 100 p.p.m. Application of naphthoxyacetic acid resulted in an increase in photosynthesis. The variations in rates of apparent photosynthesis following treatment with these substances could not be explained on the basis of changes in rate of respiration in the beans.

The work to be reported in this paper was carried out in an attempt to discover whether results similar to those reported for beans would be obtained when the same growth substances were applied to an aquatic plant without stomates.

Material and methods

Pieces of the waterweed, *Anacharis canadensis* (Michx.) Planch., were used in all experiments. Terminal branches, approximately 6 inches in length, were selected for uniformity in size and appearance from plants growing in an aquarium in the laboratory. The rates of photosynthesis and respiration were determined for each branch before any was treated in order to establish their relative performance before treatment.

Photosynthesis was measured in terms of the amount of oxygen evolved, as determined by the Winkler method, when the plants were submerged in 100-ml. test tubes filled with water containing small amounts of potassium bicarbonate, as described by MEYER and ANDERSON (3). Photosynthesis was determined for 30-minute periods, and the results were corrected for respiration which was measured subsequently. A water-bath temperature of approximately 25° C. was used for tests for both photosynthesis and respiration. The 200-watt incandescent Mazda lamp which was used as a source of light during photosynthesis provided 1000 foot-candles intensity at the location of the plants in the water bath.

Respiration was determined on the basis of the amount of oxygen used from the circumambient water. The procedure was essentially the same as for photosynthesis except that noncarbonated but well-aerated water was used and no light source was provided. Each measurement was for a 2-hour period. All data were calculated for a standard unit of time of 1 hour. Each experiment was repeated four or more times, and the results presented are based upon the means of two or more experiments in each case.

Four growth substances—2,4-dichlorophenoxyacetic, gamma-(indole)-butyric, indole-3-acetic, and beta-naphthoxyacetic acids—were tested for their effects upon photosynthesis and respiration. Each compound was first dissolved in a small amount of ethanol and then dispersed in sufficient water to give concentrations of 30 p.p.m. and 100 p.p.m. Each solution was made up fresh before being used. The sprigs of *Anacharis* were treated by immersing them in the desired concentration of a growth substance for a period of 2 hours. They were then rinsed in tap water and immediately subjected to measurements of photosynthesis and respiration along with control plants. The measurements were repeated after 24 and 48 hours. During the intervals between measurements the plants were placed in separate beakers of tap water in diffuse light near a laboratory window.

Results

2,4-D.—Treatment of *Anacharis* with 2,4-D resulted in a decrease in the rate of photosynthesis for both concentrations used, but the effects appeared sooner and were more marked at the higher concentration (table 1). In either case the amount of retardation increased with time and amounted to approximately 45% after 24–48 hours, depending upon the concentration (fig. 1). By the end of 48 hours the plants treated with 100 p.p.m. were often beginning to lose their chlorophyll and show deterioration.

Immediately after treatment the rate of respiration was lessened. At 30 p.p.m. the drop amounted to an average of 8%, but after 48 hours the plants had returned to approximately the same respiratory rate as before treatment (fig. 2). At 100 p.p.m. the effects were more striking, and immediately after treatment respiration decreased 42%. During the ensuing 48 hours there was considerable but not complete recovery (fig. 2).

INDOLE-BUTYRIC ACID.—Photosynthe-

sis declined following treatment with indole-butyric acid at both concentrations (table 1). At 30 p.p.m. the decrease amounted to 13–23% (fig. 1), becoming progressively greater with time. At 100 p.p.m. indole-butyric acid had a rather drastic effect in that the plants became limp, and photosynthesis almost, if not entirely, stopped. The experiment was repeated many times, and usually no photosynthesis could be detected following treatment with the higher concentration, i.e., apparent photosynthesis was negative, and photosynthesis, corrected for respiration, was approximately zero.

Both concentrations of indole-butyric acid caused an immediate but more or less temporary decrease in respiration (table 1, fig. 2). Forty-eight hours after treatment the rate of respiration was practically the same as before treatment.

INDOLE-ACETIC ACID.—At a concentration of 30 p.p.m. of indole-acetic acid, a slight retardation of photosynthesis, amounting to 9%, occurred immediately and persisted throughout the experiment (table 1, fig. 1). Photosynthesis was greatly reduced following treatment with 100 p.p.m. of this substance. The decrease was of the order of 50–80% for 1–48 hours after treatment (fig. 1).

At both concentrations used a temporary acceleration of respiration took place immediately after the acid was applied to the plants (table 1, fig. 2). The magnitude of the acceleration was greater for the lower concentration. After 24 hours the situation was reversed in that respiration was depressed by both treatments. The latter effect was even more pronounced after 2 days (fig. 2).

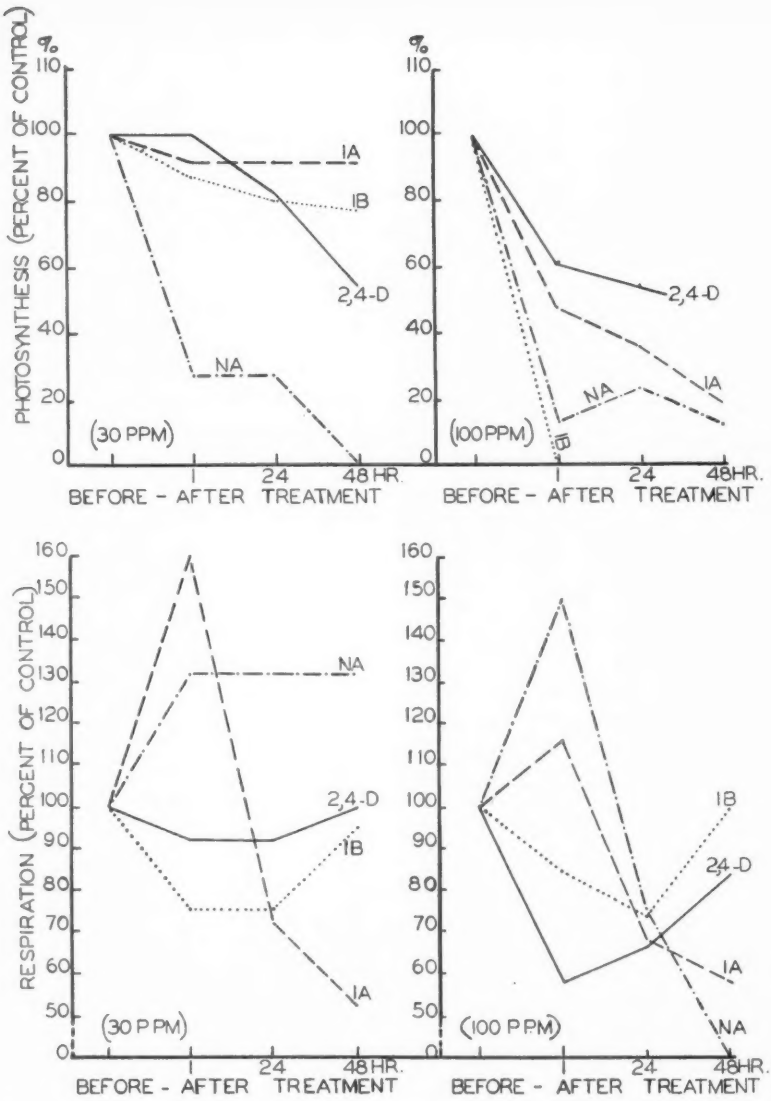
NAPHTHOXYACETIC ACID.—This substance affected the process of photosynthesis in *Anacharis* adversely (table 1, fig. 1). The effect was evident immediately after the plants were treated, amounting to a reduction of more than 70%, being somewhat more at the higher concentration. For the duration of the experiment there was little or no evidence of recovery.

TABLE 1

RATES OF PHOTOSYNTHESIS AND RESPIRATION IN ANACHARIS BEFORE AND AFTER TREATMENT WITH GROWTH SUBSTANCES, RELATIVE TO UNTREATED, CONTROL PLANTS. C, CONTROL PLANTS; T, TEST PLANTS BEFORE AND AFTER TREATMENT

TIME RELATIVE TO TREATMENT	RATIO T/C			
	Photosynthesis		Respiration	
	30 p.p.m.	100 p.p.m.	30 p.p.m.	100 p.p.m.
	2,4-Dichlorophenoxyacetic acid			
Before	1.10	1.10	1.20	1.66
1 hour after	1.10	0.67	1.10	0.96
24 hours after	0.90	0.60	1.10	1.10
48 hours after	0.60	.....	1.30	1.40
	Indole-butyric acid			
Before	0.91	0.74	1.58	0.78
1 hour after	0.79	0.00	1.20	0.67
24 hours after	0.73	0.00	1.23	0.58
48 hours after	0.70	0.00	1.50	0.80
	Indole-acetic acid			
Before	1.10	1.07	1.50	1.20
1 hour after	1.00	0.51	2.40	1.40
24 hours after	1.00	0.40	1.10	0.80
48 hours after	1.00	0.20	0.80	0.70
	Naphthoxyacetic acid			
Before	1.10	0.80	0.75	1.00
1 hour after	0.30	0.10	1.00	1.50
24 hours after	0.30	0.20	1.00	0.75
48 hours after	0.00	0.10	1.00	0.00

At the same time both concentrations of this growth substance resulted in an



FIGS. 1–2.—Rates of photosynthesis and respiration in *Anacharis*, as percentages of untreated controls, before and after treatment with growth substances at concentrations of 30 and 100 p.p.m. 2,4-D, 2,4-dichlorophenoxyacetic acid; IB, indole-butyric acid; IA, indole-acetic acid; NA, naphthoxyacetic acid. Fig. 1 (*above*), photosynthesis. Fig. 2 (*below*), respiration.

immediate acceleration of respiration (table 1, fig. 2). At a concentration of 30 p.p.m., the increase amounted to approximately 33% and persisted for the duration of the experiments. The increase following treatment with 100 p.p.m. was temporary, followed by a rapid decline until at the end of 48 hours the plants were usually starting to deteriorate.

Discussion

From the results published previously (1) regarding the general depression of photosynthesis in bean plants following treatment with various growth substances, there remained the possibility that the effects might result in part from stomatal behavior following application of the compounds. It was primarily for this reason that astomatous *Anacharis* was chosen for this study. Two concentrations of each substance were used to discover whether similar results followed the application of both a high and a low concentration.

The rate of photosynthesis was reduced in *Anacharis* treated with 2,4-D, indole-butyric acid, indole-acetic acid, and beta-naphthoxyacetic acid at concentrations of 30 and 100 p.p.m. The degree of retardation was much more marked at the higher concentration in all cases. Since similar trends in photosynthesis were obtained with the lower concentration of each growth substance as with the higher concentration, it seems unlikely that the effects resulted from general toxicity or injury to the plants. The use of astomatous *Anacharis* eliminated any possible effects that growth substances may have upon stomatal behavior. The data for photosynthesis were corrected for measured rates of respiration. Variations in the rates of respiration would not thus appear to offer any satisfactory explanation for the retardation of photosynthesis by these growth substances. Except for naphthoxyacetic acid, the results for photosynthesis in *Anacharis* agree with the data for photosynthesis in beans treated with the same substances (1). In beans treated with naphthoxyacetic acid, photosynthesis was accelerated, while in *Anacharis* no stimulation was found.

The rates of respiration in *Anacharis* after treatment with these growth substances in general show trends similar to those reported for other plants. Respiration in *Anacharis* treated with 2,4-D was reduced. The reduction was only temporary at a concentration of 30 p.p.m., but at 100 p.p.m. it was of greater magnitude and persisted for 2 days. MITCHELL *et al.* (5), working with bean roots and tomato stems, and KELLY and AVERY (2), using pea and oat tissues, reported similar effects for 2,4-D at the higher concentrations. The latter investigators obtained accelerated respiration with concentrations lower than 100 mg. per liter. No such stimulation was noted for *Anacharis*. In the present study indole-butyric acid caused an immediate decrease in respiration which lasted approximately 24 hours, the higher concentration being somewhat more effective. By the end of 48 hours there was little or no depression of respiration. MITCHELL *et al.* (5) found that indole-butyric acid brought about a decrease in respiration in tomato-stem slices. Indole-acetic acid treatment at concentrations of 30 and 100 p.p.m. caused an immediate acceleration of respiration in *Anacharis* followed by a decline 24 and 48 hours after its application. The amount of acceleration was much more pronounced with the lower concentration of this substance. These results are in agreement with data for other plants investigated by MITCHELL *et al.* (5) and others who have found

that the application of indole-acetic acid may either retard or accelerate respiration, depending upon such factors as kind of plant, concentration, pH, and oxygen tension. The data regarding respiration in *Anacharis* subsequent to the application of naphthoxyacetic acid are similar in many respects to those from the indole-acetic acid tests, especially at the higher concentration where there was immediate stimulation followed by depression. The chief difference between the effects of these two substances appeared at the lower concentration, where naphthoxyacetic acid induced an immediate increase in respiration which persisted for at least 2 days.

Summary

1. Photosynthesis and respiration were measured for *Anacharis canadensis* before and after treatment with 2,4-dichlorophenoxyacetic, gamma-(indole-3)-*n*-butyric, indole-3-acetic, and beta-naphthoxyacetic acids.

2. At concentrations of 30 and 100 p.p.m. of each substance, the rate of photosynthesis decreased, with the higher concentration being more effective.

3. The effects upon respiration were more varied. With the application of naphthoxyacetic acid at the lower concentration, a prolonged acceleration of respiration was observed, while at 100 p.p.m. there was an immediate increase followed by a depression.

4. An immediate temporary acceleration in oxygen utilization, followed by a retardation, resulted from the application of indole-acetic acid at both concentrations.

5. In the experiments with both indolebutyric acid and 2,4-D, at either 30 or 100 p.p.m., there was first a decrease in the rate of respiration, followed by partial or complete recovery by the end of 48 hours.

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ANATOMY OF THE COTTON LEAF AND EFFECTS INDUCED BY 2,4-DICHLOROPHENOXYACETIC ACID

DANIEL F. BURTON

Introduction

In the spring of 1947, Dr. O. A. LEONARD, plant physiologist at the Agricultural Experiment Station, Mississippi State College, called the author's attention to damage to cotton leaves which resulted from sprays containing 2,4-dichlorophenoxyacetic acid (2,4-D) drifting from near-by fields onto the plants. There were indications that cotton was especially sensitive to 2,4-D. Therefore, a smaller amount of 2,4-D was applied to each cotton plant in the following experiment than had been applied to each bean plant in experiments previously described (3). The experiment was designed to determine the histological development of the lamina of the normal cotton leaf and to compare the development and appearance of these leaves with those from plants treated with 2,4-D.

Material and methods

On June 11, 1947, seeds of the Stoneville 2B strain of Upland Cotton were planted in flats containing fertile loam soil, placed on a bench in the University of Chicago greenhouses. On July 2 a mixture of 0.2% 2,4-D in lanolin was applied to the upper surface at the base of the nearly expanded laminae of some of the cotyledons, in amounts estimated to be 40 mg. of mixture per plant (2). This amount contained approximately 80 μgm. of the acid. The remaining plants were left untreated as controls.

On August 27, 1947, photographs were taken of fresh material, and buds and leaves in various stages of development were collected and fixed in Navashin's solution. Fixed material was handled according to the tertiary butyl alcohol method, sectioned at 6–10 μ, and stained in Kraus's modification of Fleming's triple stain.

Observations

DEVELOPMENT AND APPEARANCE OF NORMAL LEAVES.—The leaves of Upland Cotton are simple and weakly three-lobed (fig. 1*C*). The lamina has a fine network of anastamosing veins, and there are a midrib and two additional large prominent veins in each leaf. A pair of stipules appears at the base of each petiole.

Cross sections of young leaves in the bud show a subepidermal marginal histogen that gives rise to three layers of cells within the embryonic epidermis (fig. 2*B*). Some of the cell divisions in the lower internal layers give rise to cells in planes vertical to the major plane of the lamina, thus increasing the number of internal layers to four. The four internal layers of the embryonic lamina plus the outer two layers make up the plate meristem (5). The six layers are clearly evident in a young leaf just prior to expansion (fig. 2*E*). The upper and lower layers are transformed into upper and lower epidermis. The adaxial internal layer becomes a palisade layer, and the three abaxial internal layers become spongy mesophyll. The cells of the middle layer of the latter three contain material that stains deeply with safranin in leaves that are about to expand (fig. 2*E*). In unstained sections this material appears as hyaline droplets. No similar formation is

visible in cross sections of younger leaves (fig. 2*A*) or in sections of mature leaves (fig. 4*A*).

Initial divisions in the formation of minor veins occur in a row of cells in the layer of meristematic spongy mesophyll next to the meristematic palisade. The first division produces cells in a plane vertical to the plane of the plate meristem (fig. 2*C*). In the illustration an adjacent cell has become involved in vein formation and has divided obliquely. Additional divisions of the initial cells and adjacent cells in the same and adjoining layers occur in several planes. Eventually a mass of cells, each cell smaller than those of the surrounding mesophyll, develops (fig. 2*D*).

Scattered irregularly through the mesophyll and the parenchyma of large veins of mature leaves are prominent black spheres. The spheres are hollow, and cross sections reveal hyaline material in the central cavity (fig. 3*C*). The first stage of sphere development is the formation of a single, enlarged cell in the plate meristem (fig. 3*A*) or in the parenchyma of larger veins. Later, a rounded group of several cells develops (fig. 3*B*). The cells of the group have a darker-staining cytoplasm than those of the surrounding mesophyll or vein parenchyma. At the center of the group, the cells separate and blacken. The blackening and separation spread peripherally until the black, amorphous sphere and the hyaline cavity are formed.

The lamina of the mature leaf has a

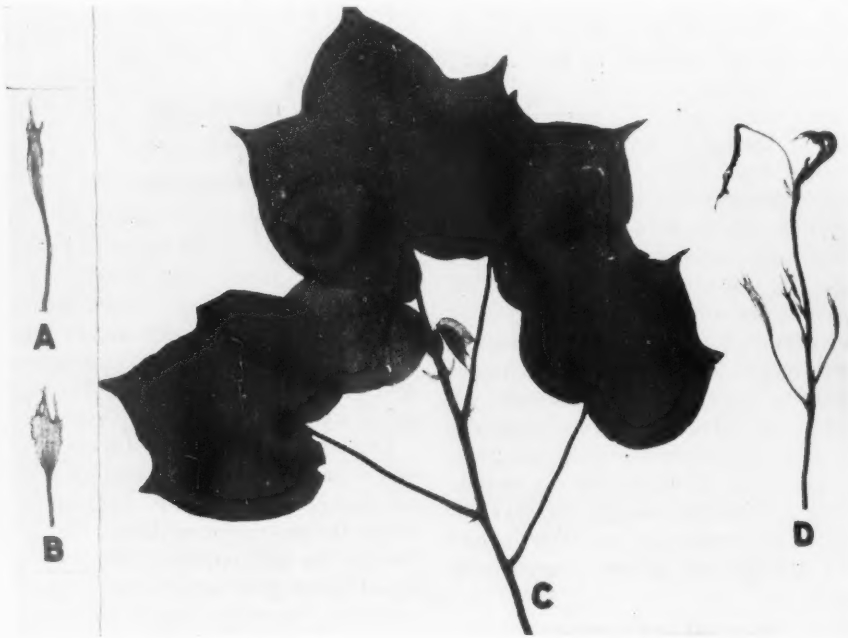

FIG. 1.—Photographs taken by transmitted light. *A*, *B*, leaves from plants treated with 2,4-dichlorophenoxyacetic acid spread flat to demonstrate narrow laminae and restricted amount of dark-appearing chlorenchyma. *C*, control plant. *D*, treated plant showing rolled and wrinkled appearance of leaves.

single, well-defined palisade layer next to the upper epidermis and an extensive spongy mesophyll between the palisade and the lower epidermis (fig. 4*A*). The midrib and prominent principal veins have extensive parenchyma around the vascular elements. Smaller veins have a less extensive sheath.

RESPONSES TO TREATMENT WITH 2,4-D.—Cotton plants treated with 2,4-D showed epinasty and curvature of the hypocotyl on the day after treatment. No other effect was visible for several days, and then leaves began to appear. All the leaves were uniformly narrow and straplike (fig. 1D). Green color appeared only in islets in the central portion of the leaf and along the rolled and

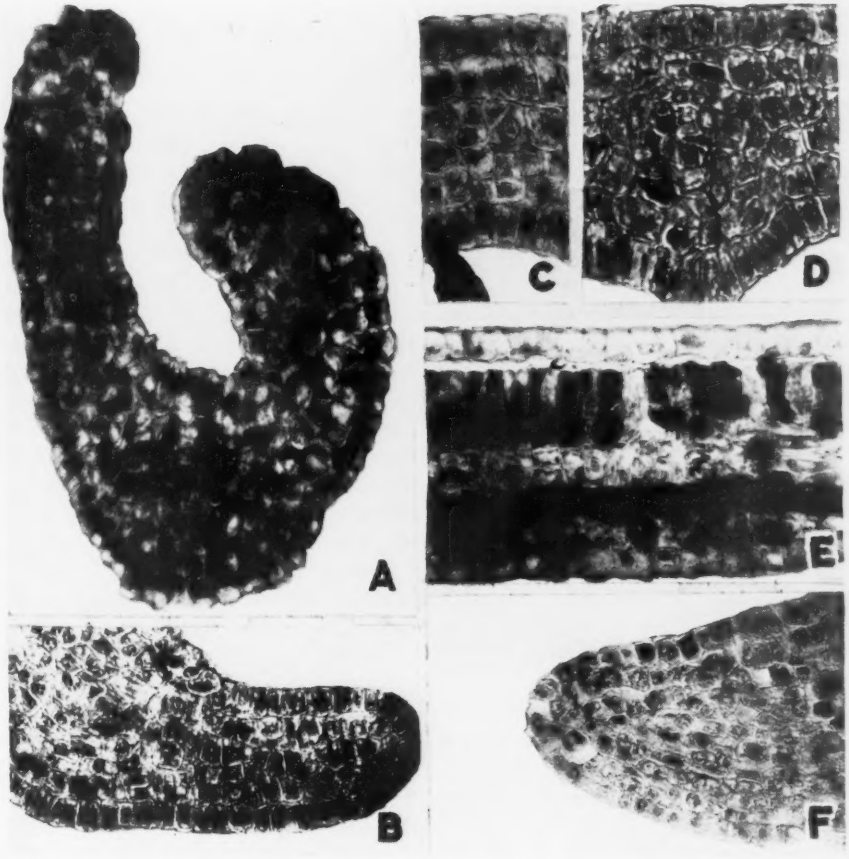

FIG. 2.—Stages of leaf development. *A*, cross section of young leaf from control plant. *B*, detail of origin of internal layers of plate meristem from marginal histogen in normal leaf; divisions in two abaxial internal layers have produced a third layer. *C*, divisions in upper layer of spongy mesophyll initiating normal minor vein development. *D*, later stage in minor vein development. *E*, lamina of normal leaf just prior to expansion; dark-staining layers appear in central of three spongy mesophyll layers. *F*, margin of young leaf from treated plant illustrating severely limited activity of marginal histogen and plate meristem.

wrinkled margin (fig. 1*A*, *B*). The green tissue photographed darker than the vein tissues by transmitted light. The three lobes of the normal leaf were represented by three prolonged teeth on the tips of

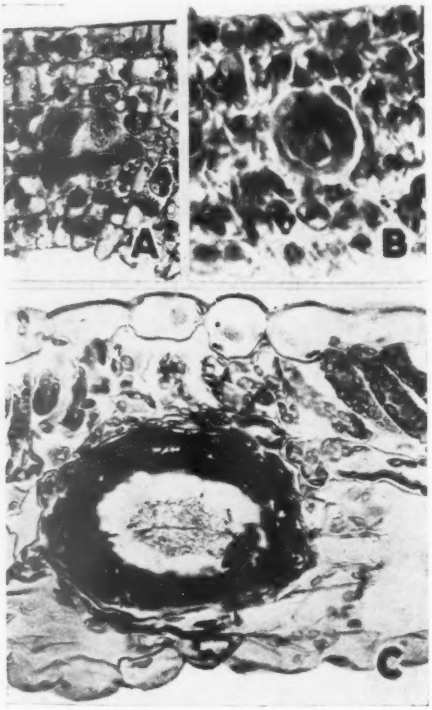

FIG. 3.—Development of cavities in normal leaf *A*, enlarging cell in plate meristem of young leaf. *B*, group of cells with darkly staining cytoplasm. *C*, cavity with black, amorphous periphery and hyaline center.

leaves from the treated plants. A pair of stipules developed at the base of the petioles of the treated plants as they do on controls.

Cross sections of buds of treated plants showed that the activity of the subepidermal marginal histogen and of the plate meristem was sharply limited in the young leaves (fig. 2*F*). Development of clearly layered plate meristem was suppressed, and the principal veins remained close to the midrib (fig. 4*B*). The parenchyma surrounding the veins formed a continuous sheet across the central portion of the leaf in the final stage of development. Between the principal veins and the midrib, immediately below the upper epidermis, were some chlorenchymatous cells containing darkly staining chloroplasts. Similar but less extensive islets of chlorenchyma were found immediately within the lower epidermis. The chlorenchyma was developed slightly more at the margin.

The course of development of the black spheres with hyaline cavities in leaves of treated plants was like that observed in control plants.

Discussion

The normal development of the lamina of the cotton leaf is strikingly similar to the development of the tobacco leaf lamina as described by AVERY (**1**). Minor veins in the anastamosing network are initiated in the layer of plate meristem of the normal leaf immediately beneath the meristematic palisade, as minor veins originate in the laminae of other plants (**1, 3, 4**).

Gross responses of cotton plants to 2,4-D resembled the responses previously described for Red Kidney bean (**2, 6, 7**).

ZIMMERMAN and HITCHCOCK (**8**) sprayed the tips of young tobacco plants with 4-chlorophenoxyacetic acid and obtained a subsequent series of leaves each of which was more modified than its predecessor. WATSON (**7**) described similar differences in the responses of Red Kidney bean leaves to treatment with 2,4-D. He used a hypodermic needle to inject 0.01 ml. of an aqueous solution containing 10 μgm. of 2,4-D and 0.5% Carbowax into the midrib of one primary leaf of each treated plant. Leaves that

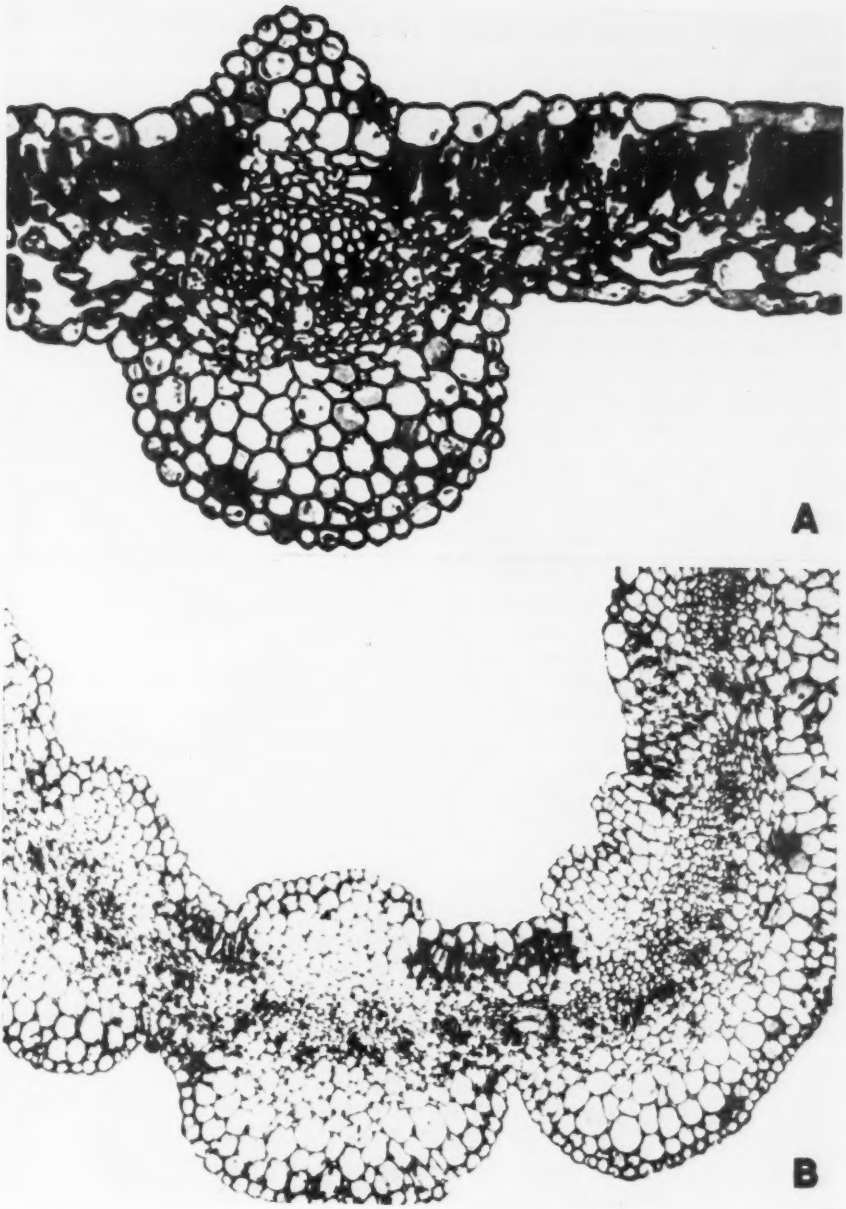

FIG. 4.—*A*, cross section through mature leaf of control plant showing well-developed mesophyll with distinct palisade and extensive spongy parenchyma. *B*, cross section through fully developed leaf of treated plant; veins are fasciated and chlorenchyma is restricted to islets immediately within epidermis.

were younger at the time of treatment showed progressively less expansion of the lamina as they developed. After the most modified of these leaves developed, a series then appeared that showed gradual recovery of the normal growth pattern. WATSON correlated the stages of development at the time of treatment and the degree of sensitivity to formative modification by 2,4-D. He found that the most critical stage was one prior to cell differentiation in which the cells were isodiametric and filled with cytoplasm. The author of the present paper has observed a like effect of 2,4-D on Red Kidney bean leaves (3). A 0.5% concentration of 2,4-D in a carrier was applied to the upper surface of the base of the laminae of the primary leaves of treated plants. The amount applied has been estimated to be 40 mg. of the mixture per plant (2) and to contain 200 μgm. of the acid. Following treatment, each successively formed leaf had smaller and more straplike leaflets than the preceding one. None of the plants developed a series of leaves showing recovery, but the experimental technique differed from that used by WATSON. The writer applied a greater quantity of 2,4-D per plant, and this may have caused sufficient damage to prohibit recovery. The external application apparently spread the absorption of the compound over a longer period than the injection method, thus affecting more leaves at the most critical stage of development.

The leaves of cotton plants, unlike bean leaves, gave uniform responses to the application of 2,4-D, and all developed into straplike forms closely resembling the most modified bean leaflets. At the time of treatment no leaves were visibly developed from the terminal bud of the cotton plants, while the first trifoliate leaf of bean was apparent at the time of treatment (3). Apparently all the cotton leaves were young enough to be affected at the stage of maximum sensitivity. No signs of recovery were observed in later stages of the experiment, although cotton has been observed to recover from accidental damage by 2,4-D. The amount of 2,4-D applied may have been sufficient to prevent recovery, or recovery may have been delayed beyond the time of the experiment, because the absorption of the 2,4-D occurred over a long period of time.

Summary

1. The marginal histogen of the cotton leaf produces three internal layers of plate meristem. The adaxial of the three layers becomes the palisade layer of the mature leaf. Divisions producing cells vertical to the major plane of the leaf lamina increase the two abaxial layers to three layers that become spongy mesophyll.

2. Minor veins are initiated by divisions in a row of cells in the adaxial of the spongy mesophyll layers. The first division of one of these cells produces cells in a plane vertical to the plane of the plate meristem. Adjacent cells in the same and adjoining layers become involved in vein formation.

3. The black, hollow spheres in vein parenchyma and in mesophyll originate with the enlargement of single cells. Each cell becomes a group of cells with darker-staining cytoplasm. The group of cells develops into a black hollow sphere with a hyaline central cavity.

4. Treatment of the expanded laminae of the cotyledons of young plants with approximately 40 mg. of a 0.2% concentration of 2,4-dichlorophenoxyacetic acid in lanolin inhibited activity of the marginal histogen and of the plate meristem in successively formed leaves. Major veins became fasciated. Chlorenchyma-

tous tissue developed only in islets in the central portion of each leaf and along its margin.

The writer wishes to express his appreciation to Dr. GEORGE W. JOHNSTON of the Department of Botany, Mississippi State College, for the seed of the Stoneville 2B cotton used in this experiment, and to Dr. JOHN F. LOCKE of the same department for information concerning the literature on cotton.

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GROWTH AND DEVELOPMENT OF BUCKWHEAT UNDER DIFFERENTIAL TEMPERATURE GRADIENTS

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Introduction

Temperature is a major factor in regulation of the developmental cycle in plants, and numerous studies have been made of its effects upon plant ontogeny. Most of them have involved the technique of exposing entire plants to different or varying temperatures. Such procedures make it difficult, if not impossible, however, to determine interrelationships among parts or to identify the particular organs through which the primary effects of contrasted temperatures are exerted. In an attempt to clarify certain reciprocal effects of roots and shoots during ontogeny, an experiment was undertaken in which the aerial and subterranean parts of plants were maintained at considerably different temperatures. Developmental responses of individual plants whose roots and shoots were maintained at contrasted temperatures were compared with controls in which entire plants were grown under uniformly high or low temperature conditions, respectively. Formative responses of the various temperature series were followed throughout the life-cycle.

Material and methods

The Japanese variety of common buckwheat (*Fagopyrum esculentum*) was selected as a rapidly maturing annual whose development is characterized by readily recognizable growth stages. Because buckwheat is photoperiodically indeterminate in its reproductive responses, its use for temperature studies

seemed especially appropriate in view of the usual sensitivity of flowering in day-neutral types to temperature. The erect, uniaxial habit of buckwheat also facilitates quantitative measurements of its growth rate.

The experiment was performed under greenhouse conditions from February 18 to April 5. Seeds were sown February 18 in flats of sand and germinated at ordinary temperatures (18°–21° C.). On February 22 four seedlings were transplanted to each 2-gallon nutrient culture jar filled with a uniform weight of solute-free quartz gravel. Immediately following transplanting, uniform temperatures (18° C.) were maintained temporarily throughout all parts of the experiment until February 24 when it was considered that the seedlings were satisfactorily established. The plants were then divided into four different temperature series and subjected to the following treatments: (*a*) shoots and roots maintained at 13° C., designated as series L/L; (*b*) shoot temperature at 13° C., roots at 21° C., designated as series L/H; (*c*) shoots and roots both maintained at 21° C., designated as series H/H; and (*d*) shoot temperature at 21° C., roots at 13° C., designated as series H/L. Data presented thus start with the beginning of differential temperature treatments on February 24 and end on April 5, when the experiment was terminated, covering a total experimental period of 40 days.

Jars of series L/H were incased in an electrically heated, thermostatically controlled, insulated bench with the thermostat set at 21° C. Series L/L was placed on adjoining benches in the same room, and the desired room temperature of 13° C. was maintained by radiators and regulation of ventilators. A similar arrangement was employed for the converse series in an adjoining room except that jars of series H/L were incased in an insulated refrigerated bench, maintained at 13° C. by a thermostat. The thermostatic control of the radiators was set at 21° C., and ventilators were regulated as necessary to keep the room temperature at 21° C. Air within the rooms was circulated by electric fans to aid in maintaining uniform room temperatures. The 8-degree transition in temperature of series L/H and H/L in the insulated benches thus occurred at the ground line, so that their roots and shoots were kept at contrasted temperatures throughout the experiment. Small electric fans kept the air in constant circulation within these benches and aided greatly in the maintenance of uniform root temperatures. Throughout the experiment the 8-degree temperature differential between roots and shoots in H/L and L/H was maintained with the exception of the last 5 days of March when air temperatures were unseasonably high. The differentials, however, were never less than 6°.

Continuous temperature and humidity records were kept by means of thermographs and hygrographs placed within the rooms and insulated benches. Light intensity varied considerably during the experiment, with the average intensity at noon being about 1,000 foot-candles. The low intensities of natural daylight on cloudy days were supplemented with a battery of 40-watt fluorescent "daylight" lamps giving an intensity of approximately 200 foot-candles at the bench level.

All plants were grown on WITHROW's nutrient solution for moderate light (18). A 0.1% concentration of total solutes was employed until the plants were about 1 foot high and then gradually shifted to 0.2%. Sixteen days after the initiation of temperature treatments, symptoms of nitrogen deficiency were noted in series

L/L, L/H, and H/L. Thereafter 2.5 gm. of ammonium nitrate were added to each 20 liters of nutrient solution. The jars were completely drained and the solution renewed in uniform amounts once a week until the plants began to enlarge rapidly about 20 days after the start of temperature treatment; thereafter, twice a week. The original nutrient solution was adjusted to pH 6.5, and the pH values of the leachates were determined electrometrically.

A growth record was maintained from the beginning of temperature regulation (February 24) until the termination of the experiment (April 5), with measurements and observations at 4-day intervals. All measurements were recorded not only by date but also in terms of the phase of development.

Absolute fresh and dry weights were determined from a composite sample of eighteen plants per series at five different developmental stages throughout ontogeny. Roots were separated from shoots, carefully washed free of gravel in distilled water, and quickly dried between sheets of absorbent paper to remove external moisture. Fresh weights of roots and shoots were determined gravimetrically, the parts comminuted, and triplicate samples were weighed for dry weight in a Brabender moisture tester. Ash was determined only at the final sampling by slow ignition of dried tissue over the open flame of a Bunsen burner, and weighing to constant weight.

Results and discussion

GENERAL OBSERVATIONS.—During the first 2 weeks following the initiation of temperature treatment all series followed a fairly uniform pattern of development. Good secondary roots had formed in all series except L/H. After the second week of treatment, however, pronounced differences in stem diameter, rates of stem elongation, leaf size, color, and root systems became apparent among the various series (tables 1, 2).

Average daily water consumption rose rapidly in all series during early vegetative growth, with series H/H showing the highest average consumption per plant (table 3). During late vegetative growth and incipient formation of flower buds a marked decline in water intake became evident in all groups, absorption per plant falling to less than half the daily mean of the earlier vegetative period. When computed on a leaf-area basis, the decline in water consumption at this time was even more accentuated, even though the average leaf area per plant had increased in most cases by tenfold between the two stages (fig. 3). In all cases a slight increase in water absorption per plant (but not on a leaf-area basis) was noted during late budding and anthesis. The peak of water intake per plant occurred at the time of seed formation. The water curves show a similar trend in all series, suggesting a natural ontogenetic "rhythm" of water absorption irrespective of experimental treatment (table 3). Early vegetative and fruiting stages were comparable in terms of water consumption.

On the basis of pH changes of leachates, differences in metabolic activity became evident among the series at an early date (fig. 1). Irrespective of temperature there was a general tendency of leachates to become acid during the flowering stage. Plants in series H/H were consistently the most vigorous (fig. 6, tables 1, 2, 4). Leachates from this series showed a reduction in pH value at the earliest measurement. This was in contrast to series L/H, which showed an initial rise in pH value followed by a gradual reduction. Series L/L and H/L were inter-

mediate in hydrion changes of residues, showing a rise in pH values at the earliest measurements but diminishing after the late vegetative stage. These changes in pH values of nutrient solutions in the different series showed a consistent correlation with the development of the root systems and growth in general. Periods of most vigorous growth were associated with maximum acidity of nutrient leachates, and the greatest alterations in substrate pH were correlated with periods of maximum water absorption (*fig.* 1, table 3).

Large shifts in top-root ratios indicated significant differences in rates of shoot and root growth during a given stage of development. Root growth was often static while shoots were enlarging (table 4). There was little gain in root dry weight during budding or early flowering; the greatest increase occurred late in ontogeny. Correlation of temporary cessation of root growth with impaired uptake of nutrients and draining of reserves from other organs during the metamorphosis of floral development has been cited repeatedly by other workers (**1, 3, 6, 10, 11**). ROBERTS AND STRUCKMEYER (**17**) concluded that the composition and the reserve conditions of the shoot were largely the controlling factors in the production of roots.

The stimulus of syngamy (**13**) was re-

TABLE 1

AVERAGES FOR STEM DIAMETER, TOTAL STEM LENGTH, AND DAILY STEM LENGTH INCREASE IN CENTIMETERS

Age in days	Growth stage	Stem diameter*	Stem length	Daily increase	Growth stage	Stem diameter*	Stem length	Daily increase
	Series L/L				Series L/H			
4	Veg.	0.18	5.2	1.30	Veg.	0.17	4.8	1.20
8	Veg.	0.20	6.3	0.26	Veg.	0.20	5.3	0.13
12	Veg.	0.22	7.4	0.26	Veg.	0.20	6.0	0.18
16	Veg.	0.26	8.5	0.29	Veg.	0.21	7.6	0.40
20	Bud	0.27	10.3	0.45	Veg.	0.22	8.2	0.15
24	Fl.	0.30	15.3	1.25	Bud	0.23	10.5	0.58
28	Fl.	0.32	20.1	1.20	Fl.	0.25	13.1	0.65
32	Fl.	0.35	23.8	0.93	Fl.	0.31	15.6	0.63
36	Fl.	0.38	33.5	2.42	Fl.	0.33	19.4	0.95
40	Fr.	0.40	38.6	2.30	Fl.	0.35	21.5	0.52
	Series H/H				Series H/L			
4	Veg.	0.18	5.1	1.30	Veg.	0.19	5.1	1.30
8	Veg.	0.20	6.4	0.33	Veg.	0.20	5.7	0.15
12	Veg.	0.34	10.7	1.08	Veg.	0.28	8.1	0.60
16	Bud	0.36	15.5	1.20	Bud	0.29	9.0	0.23
20	Fl.	0.45	23.3	1.95	Fl.	0.30	15.8	1.70
24	Fl.	0.48	31.3	2.00	Fl.	0.31	18.5	0.68
28	Fl.	0.50	38.5	1.80	Fl.	0.31	23.4	1.22
32	Fl.	0.53	45.2	1.67	Fl.	0.32	28.4	1.25
36	Fr.	0.54	49.0	0.95	Fl.	0.33	30.2	0.45
40	Fr.	0.55	58.4	2.35	Fr.	0.35	35.3	1.02

* Measured at basal node.

TABLE 2

AVERAGE NUMBER OF LEAVES PER PLANT, AREA PER LEAF, AND AREA PER PLANT IN SQUARE CENTIMETERS

Age in days	Growth stage	No. leaves	Area per leaf	Area per plant	Growth stage	No. leaves	Area per leaf	Area per plant
	Series L/L				Series L/H			
4	Veg.	2	3.00	6.00	Veg.	2	2.40	4.80
8	Veg.	3	3.03	9.09	Veg.	3	2.63	7.89
12	Veg.	4	8.23	32.92	Veg.	3	3.02	9.06
16	Veg.	4	11.50	46.00	Veg.	3	4.40	13.20
20	Bud	5	14.46	72.30	Veg.	4	6.43	25.72
24	Fl.	6	19.41	116.46	Bud	5	10.03	50.15
28	Fl.	7	21.50	150.50	Fl.	5	14.51	72.55
32	Fl.	8	24.00	192.00	Fl.	6	15.80	94.80
36	Fl.	8	29.06	232.48	Fl.	6	20.45	122.70
40	Fr.	10	36.31	363.10	Fl.	7	22.91	160.37
	Series H/H				Series H/L			
4	Veg.	2	3.15	6.30	Veg.	2	3.20	6.40
8	Veg.	3	7.25	21.75	Veg.	3	5.50	16.50
12	Veg.	4	14.60	58.40	Veg.	4	9.63	38.52
16	Bud	5	19.86	99.30	Bud	4	12.80	51.20
20	Fl.	7	25.57	178.99	Fl.	6	13.32	79.92
24	Fl.	9	31.20	280.80	Fl.	7	15.00	105.00
28	Fl.	12	36.50	438.00	Fl.	8	17.35	140.80
32	Fl.	13	38.38	498.94	Fl.	8	19.20	153.60
36	Fr.	17	43.25	735.25	Fl.	9	21.90	197.10
40	Fr.	19	45.50	864.50	Fr.	10	24.20	242.00

TABLE 3

AVERAGE WATER CONSUMPTION PER PLANT IN CUBIC CENTIMETERS DAILY

AGE IN DAYS	SERIES L/L		SERIES L/H		SERIES H/H		SERIES H/L	
	Growth stage	Cc. daily	Growth stage	Cc. daily	Growth stage	Cc. daily	Growth stage	Cc. daily
4	Veg.	21.00	Veg.	23.60	Veg.	25.20	Veg.	25.00
8	Veg.	34.65	Veg.	32.74	Veg.	36.70	Veg.	33.70
12	Veg.	36.50	Veg.	33.50	Veg.	14.88	Veg.	13.49
16	Veg.	13.88	Veg.	20.80	Bud	8.00	Bud	10.50
20	Bud	12.40	Veg.	16.19	Fl.	19.59	Fl.	18.90
24	Fl.	18.60	Bud.	14.00	Fl.	36.60	Fl.	21.00
28	Fl.	27.00	Fl.	27.27	Fl.	42.50	Fl.	28.30
32	Fl.	35.30	Fl.	28.00	Fl.	28.76	Fl.	14.57
36	Fl.	28.70	Fl.	28.50	Fr.	53.00	Fl.	16.60
40	Fr.	40.88	Fl.	32.00	Fr.	80.30	Fr.	36.57

flected in all series in maximum dry-weight gains of shoots between the twenty-sixth and thirty-third day (late anthesis or early fruiting stages). Dry weight of shoots during this period was increased over 50% in all series except H/L; the latter series showed a relative high rate of leaf abscission during this time. Maximum gain of root dry weight, however, increased relatively more from 33 to 40 days. This is probably accounted for by translocation from the shoots and accumulation of reserves in the root during the late ontogenetical stages. Regardless of temperature treatment, all series displayed a somewhat similar trend in *minima* and *maxima* of anabolic efficiency (dry weight per plant ÷ leaf area

TABLE 4

AVERAGE FRESH AND DRY WEIGHTS IN GRAMS PER PLANT AND PERCENTAGE DRY WEIGHT THROUGHOUT ONTOGENY; ASH WEIGHT IN GRAMS PER PLANT AT FINAL SAMPLING

Fraction	Series L/L			Series L/H			Series H/H			Series H/L		
	Shoot	Root	T/R	Shoot	Root	T/R	Shoot	Root	T/R	Shoot	Root	T/R
	14 days—vegetative stage											
Fresh wt. (gm.)	1.05	0.05	21.0	0.28	0.03	9.3	1.37	0.10	13.7	1.02	0.05	20.4
Dry wt. (gm.)	0.08	0.10	8.0	0.04	0.01	4.0	0.11	0.04	2.8	0.10	0.02	5.0
Dry wt. (%)	7.61	20.00	.....	14.28	33.30	.....	8.02	40.00	.....	9.80	40.00	.....
	20 days—budding stage											
Fresh wt. (gm.)	1.98	0.16	12.2	0.77	0.13	5.9	4.06	0.21	19.3	2.58	0.16	16.1
Dry wt. (gm.)	0.19	0.04	4.7	0.08	0.04	2.0	0.30	0.05	6.0	0.29	0.06	4.8
Dry wt. (%)	9.59	25.00	.....	10.38	30.76	.....	7.38	23.80	.....	11.24	37.50	.....
	26 days—flowering stage*											
Fresh wt. (gm.)	2.91	0.20	14.5	1.52	0.14	10.8	6.56	0.32	20.5	3.46	0.25	13.8
Dry wt. (gm.)	0.29	0.05	5.9	0.12	0.05	2.4	0.68	0.07	9.7	0.39	0.06	6.5
Dry wt. (%)	9.96	25.00	.....	7.89	35.71	.....	10.36	21.87	.....	11.27	24.00	.....
	33 days—late flowering stage											
Fresh wt. (gm.)	5.80	0.21	27.6	2.56	0.19	13.5	11.19	0.86	13.0	3.58	0.43	8.3
Dry wt. (gm.)	0.61	0.06	10.1	0.30	0.05	6.0	1.32	0.12	11.0	0.53	0.08	6.6
Dry wt. (%)	10.50	28.50	.....	11.71	26.31	.....	11.78	13.95	.....	14.80	18.60	.....
	40 days—fruiting stage†											
Fresh wt. (gm.)	5.80	0.83	6.9	3.00	0.37	8.1	11.88	2.30	5.1	3.62	0.78	4.6
Dry wt. (gm.)	0.72	0.17	4.2	0.21	0.08	3.9	1.75	0.28	6.3	0.56	0.11	5.1
Dry wt. (%)	12.40	20.48	.....	10.33	21.62	.....	14.73	12.17	.....	15.46	14.10	.....
Ash wt. (gm.)	0.09	0.02	4.5	0.05	0.01	5.0	0.30	0.05	6.0	0.07	0.08	0.8

* Budding stage in L/H.

† L/H still flowering.

per plant). Minima occurred during the inception of floral primordia and maxima during late anthesis (fig. 5).

SERIES H/H.—Within 12 days after the start of temperature regulation, plants of this group were the largest and most mature of the four series. Average stem diameter and total stem length were greater than in the other series at each subsequent date of measurement (table 1, fig. 6). By maturity the largest number of leaves (almost twice the number of any other series) with the largest aggregate leaf area was produced (table 2). Although this series produced the largest total leaf area per plant and had the greatest aggregate water consumption, examination of figure 3 reveals that with the exception of the first measurement series H/H had the lowest water consumption per unit area of leaf surface. The water requirement was also lowest in this group (fig. 4). The anabolic ratio (dry weight per plant ÷ leaf area per plant), however, was consistently the lowest of all series at the five sampling dates (fig. 5). It reached its minimum in series H/H during budding and its maximum during flowering. Although the work of other investigators suggests that the aggregate anabolic ratio may be of little significance, the correlation of minima and maxima with budding and blossoming nevertheless provides useful indexes to the metabolism at these particular stages of development. NIGHTINGALE (15) and PLITT (16) stated that with tomato and radish plants there is little or no correlation between the rate of water intake and anabolic efficiency; they found carbohydrate synthesis to be high at low temperatures.

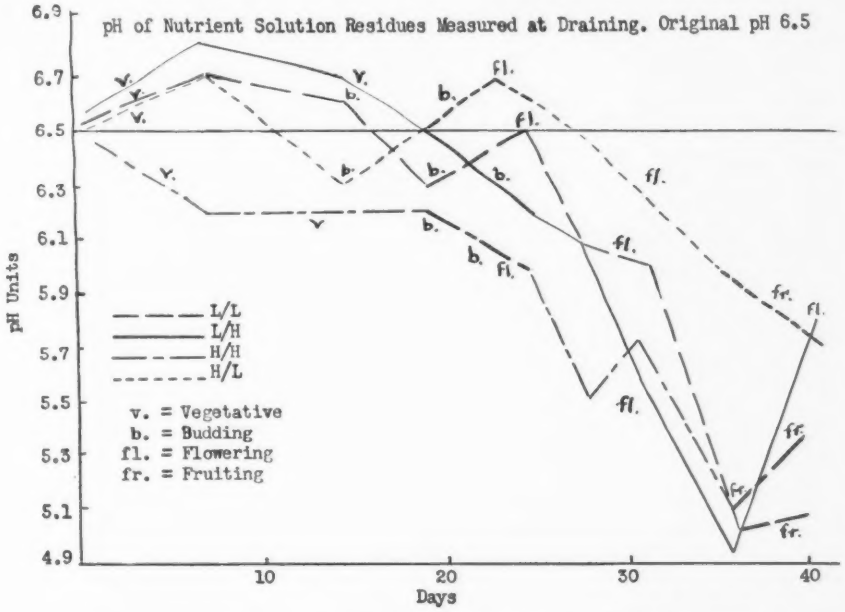

FIG. 1.—pH of nutrient leachates compared with pH of original nutrient solution

In agreement with the findings of NELSON (14), working with hemp, high soil and air temperatures had the effect of shortening the life-span. With Marquis wheat, WORT (19) similarly observed that high soil temperatures accelerated heading by as much as 11 days. In the present study with buckwheat the earliest inception of flowering and the production of the greatest number of flowers were manifested by series H/H, where the plants were exposed to high temperatures of both soil and air (fig. 2). Flowers had appeared as low as the first node by the end of the blossoming period. The greater number of flowers produced by the H/H series in contrast with other groups is striking.

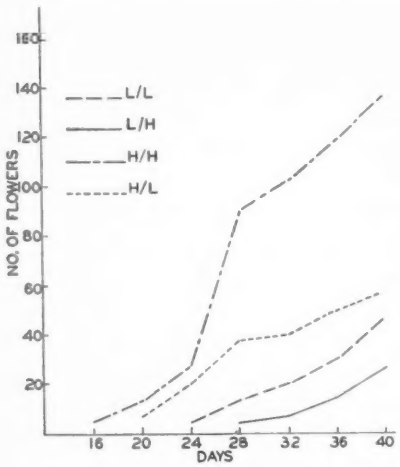

FIG. 2.—Average number of flowers per plant

Despite rapid growth and large size this series did not exhibit any signs of a transitory nitrogen deficiency with the inception of floral primordia such as occurred in all other groups. This would seem to indicate more efficient and uniform absorption and/or utilization of nitrogen at the higher temperatures of this experiment. The nitrogen deficiency in the other series at this time was the result of the relatively low level of nutrition, purposely employed in an attempt to detect evidence of reduced absorption and periods of high nutrient requirements. This response is usually not observed in plants on high nitrogen supply because of their ability to utilize nitrogen reserves if they have had an opportunity for luxury consumption in early life. In the present instance the low nitrogen supply prevented accumulation of internal reserves of nitrogen.

Total fresh, dry, and ash weights throughout ontogeny and at the final sampling were highest in this series (table 4). Examination of the distribution of the total ash (table 4), however, discloses that much larger proportions of the inorganic material of H/H had accumulated in the shoots than in the roots.

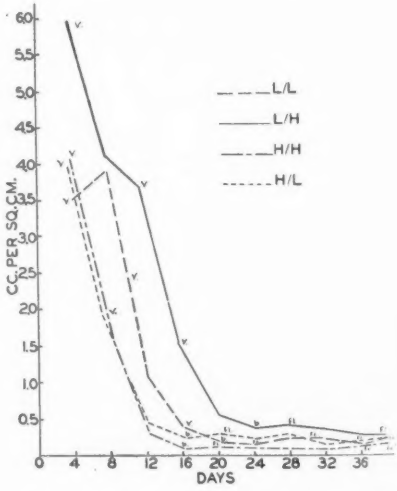

FIG. 3.—Cubic centimeters of water consumed per square centimeter of leaf area during ontogeny.

SERIES H/L.—As in series H/H flower buds appeared in the H/L series at a relatively early date. This and other signs of advanced maturity indicated

that high shoot temperatures accelerated the developmental cycle. Coincident with, or even slightly before, budding, these plants began to exhibit the effects of low root temperature (8). Leaves became chlorotic, often being rolled and wilted. Sixteen days after the initiation of temperature treatment marked symptoms of nitrogen deficiency became apparent in the series and persisted even with the additions of supplementary nitrogen as ammonium nitrate; this suggests impaired absorption and/or poor utilization of nitrogen. Nutritional difficulties were demonstrated by the variable size of the plants within the series and by drying and abscission of some of the flowers and lower leaves in the later developmental stages. These observations agree with those of BROWN (2) and CARROL (4), who found low soil temperatures to be more injurious to the development of turf grasses than low air temperatures.

During vegetative growth the mean stem length exceeded that of the two series with low shoot temperatures (L/L and L/H) but increased at an even greater rate immediately following budding (table 1). Thereafter, however, this group was slowly overtaken by the L/L series (table 1). Although consuming the lowest total amount of water during the experiment, the H/L group produced the third largest aggregate leaf area at the final measurement. Up until the time of flowering, H/L exceeded the two series with low shoot temperatures in total leaf area per plant but was surpassed by L/L in this response after this date. Low absorption owing to the low root temperature coupled with high transpiration probably accounted for some of the leaf abscission noted in the H/L series (6). The difference in leaf and stem responses between the two series with high shoot temperatures (H/H and H/L) may probably be attributed to the relative rates of absorption and assimilation of the two root systems in the contrasted temperature environments, particularly as to nitrogen and water uptake (6). There is consensus in the literature that the viscosity of water is increased at low soil temperatures and that, as a consequence,

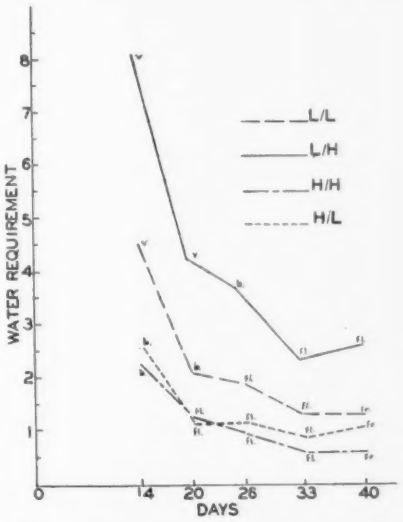

FIG. 4.—Water requirement (cubic centimeters of water, in thousands, to produce one gram of dry weight).

water is physiologically less available to the plant (9, 12). Lowered absorbing capacity of roots is also attributed, in part, to decreased permeability of the root cell walls and protoplasm (9). The water requirement in series H/L was the second lowest, following rather closely that of series H/H (fig. 4). It showed a higher anabolic efficiency than series H/H at the five sampling dates but also exhibited a minimum at budding and a maximum during late flowering (fig. 5).

The plants in this group flowered shortly after series H/H but formed only

about a third the total number of flowers of the latter (fig. 2).

Total fresh, dry, and ash weights were considerably less than in series H/H (table 4). It is noteworthy that, although the total fresh and dry weights were less than in L/L, the total ash weight exceeded L/L by 0.04 of a gram, with the greatest root ash of all groups. This suggests impaired translocation from, as contrasted with absorption of inorganic materials into, the roots; reference to the dry weight of the shoots and roots (table 4) suggests impaired translocation of reserves from shoots. In the converse series L/H, total dry weight and ash produced was much less, but salt distribution was more uniform within the plants than in series H/L (table 4).

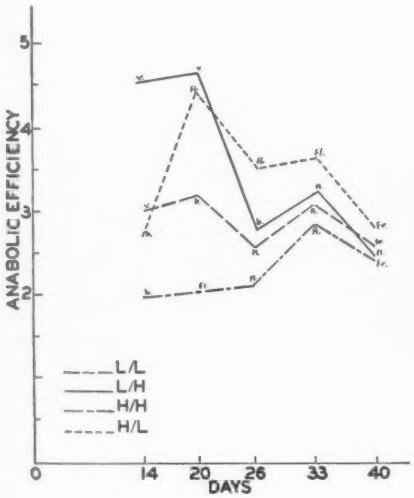

FIG. 5.—Anabolic efficiency (dry weight in milligrams ÷ leaf area in square centimeters).

SERIES L/L.—Presentation of data as a mean obscures the variation displayed by individual plants in the different growth responses of this group. In general, the plants were stocky, with relatively large leaves, and had a large aggregate leaf area though possessing relatively few leaves (table 2). In harmony with their stocky nature they exhibited comparatively large stem diameters (table 1). During the early developmental stages this series followed the two series with high shoot temperatures (H/H and H/L) in amount of stem elongation but exceeded H/L in stem height at maturity (table 1).

The effects of low shoot temperature were manifested in both L/L and L/H as being the diametric converse to the two series with high shoot temperature, H/H and H/L, in that low temperatures prolonged the life-span with increased metabolic activity in the late ontogenetic stages. Inception of flower-bud primordia and anthesis was retarded, and aggregate flower numbers were lower (fig. 2). Coincident with the relatively large leaf area the second highest total water consumption was found in series L/L in spite of the low temperature environment of both roots and shoots (table 3). The water requirement was considerably higher than that of the two series with high shoot temperatures throughout ontogeny except at the final sampling (fig. 4).

These plants produced greater absolute fresh and dry weights than series H/L and L/H at the final determinations, following series H/H in total amount (table 4), but surprisingly was third in ash weight. The shoot tissues of this group, as well as L/H, possessed a higher water content than the series with high shoot temperature, with a preponderance of both dry weight and ash in the shoots, indicating considerable storage in this region.

SERIES L/H.—In direct contrast to series H/H this group at harvest was consistently the poorest from every viewpoint. Even at an early date the plants were small, chlorotic, wilted,

showed deficiency symptoms, especially for nitrogen, and had small root systems. As in the other series with low shoot temperature, L/L, the life-cycle was prolonged with indexes of increased metabolic activity in the late phase of development, compared with the series with temperatures above 55° F. Other factors characterizing the poor growth of this group were: smallest stem diameters, lowest stem height (table 1, fig. 6), and lowest absolute, fresh, dry, and ash weights of all series during ontogeny (table 4).

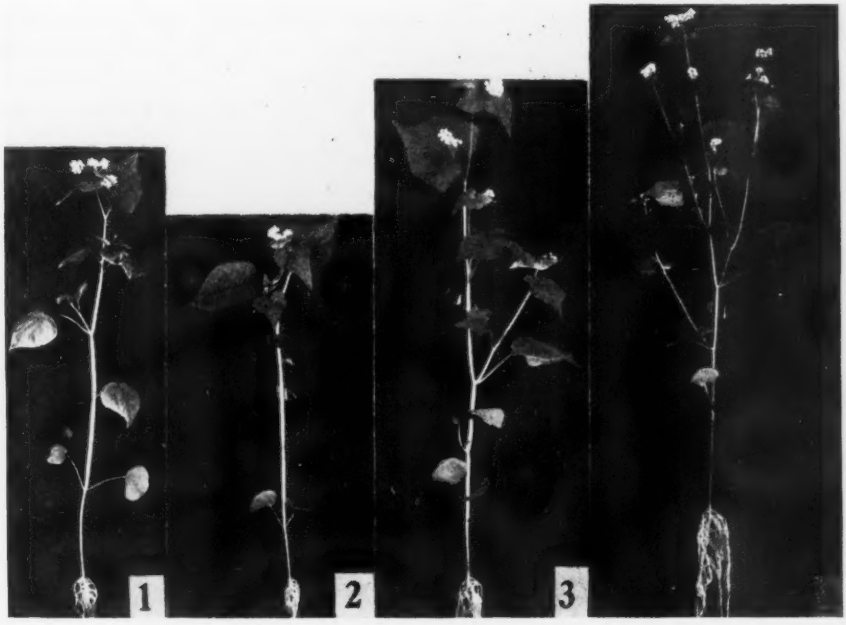

FIG. 6.—Representative plants photographed at 35 days of age. 1, series H/L. 2, series L/H. 3, series L/L. 4, series H/H.

high shoot temperature. The plants were still in the blossoming stage at the close of the study and gave indications of still being vegetative. Lowest leaf number with the corresponding smallest total leaf area per plant was produced by this temperature series throughout (table 2). The water consumption, however, was relatively high, perhaps owing to the high temperature environment of the roots, although CLEMENTS and MARTIN (5) noted the lack of correlation between transpiration in the sunflower and soil

As might be expected the water requirement was consistently the highest (fig. 4). The plants flowered almost 2 weeks after the series with high shoot temperature, and somewhat later than series L/L, and produced the lowest number of flowers (fig. 2). It is of interest that the findings of NELSON (14), working with hemp and employing a comparable temperature differential, were in direct contrast to the gross morphological and physiological characteristics observed in this study. HESSELTINE (7),

however, noted results with sunflower exposed to high root—low shoot—temperatures in agreement with data presented for buckwheat.

Summary

1. The ontogenetic cycle of common buckwheat, *Fagopyrum esculentum*, grown under greenhouse conditions in gravel cultures maintained at uniform levels of water and nutrient supply, was traced from germination to maturity. Growth and developmental responses of plants whose roots and shoots were maintained at differential temperatures were compared with controls in which entire plants were grown under uniformly high or low temperature conditions.

2. Major growth and developmental responses noted were:

a) The apparent major factor in determining the duration of the developmental cycle in buckwheat is the temperature of the air (shoot temperature).

b) High air temperatures curtailed the vegetative phase and shortened the life-span. Anthesis occurred earlier, and maturity and senescence were rapid at higher air temperature. Leaves were numerous, but the water requirement per unit of dry weight was low. High air temperature increased accumulation of inorganic material (ash).

c) Low air temperature, on the other hand, prolonged ontogeny considerably. Thus, in the series with low shoot and high root temperature, no seeds had formed when the experiment was terminated, and the plants were still growing actively. Increase in duration of the vegetative phase did not, however, result in any commensurate increase in amount of plant material produced. The percentage of ash of entire plants was less at low than at high air temperatures.

d) Water consumption followed a similar curve for all series, being the lowest at the time of floral primordia inception followed by a secondary increase concurrent with seed formation. The total amount of water absorbed was increased by high soil temperatures.

e) Changes in pH value of leachates reflected differences in rate and amount of the metabolic activity of the different series and were closely correlated with the resultant structural alterations. Rapid vegetative growth was associated with a decrease in pH value of leachates followed by a slow increase coincident with flower inception. A secondary increase in hydrion concentration accompanied anthesis and syngamy, but with the onset of maturity and senescence pH values shifted upward again.

f) A balanced temperature condition (shoots and roots at the same temperature) was more conducive to normal development of buckwheat than differential root and shoot temperatures. In terms of dry-weight yield, the treatment with the higher soil and air temperature (H/H) was most beneficial.

g) If a distinction is made between growth and development on the basis, respectively, of increase in size as opposed to rapidity of progression through the life-cycle, the shoot temperature appears primarily to regulate development and root temperature to influence chiefly the general growth processes in this particular annual.

The writer wishes to acknowledge the assistance of Dr. W. F. LOEHWING while conducting this problem under his direction at the State University of Iowa.

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BREEDING FOR INCREASED ASCORBIC ACID CONTENT IN TOMATOES[1]

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Introduction

During the past few years the attention given to vitamin content of human food and livestock feeds has increased, and the effect of environment and inheritance on the vitamin content of a plant has been tested, to a limited extent, experimentally. As would be expected

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from theoretical considerations, cases have been found where one or both factors are important. The effect of environmental and genetic factors is dependent upon many factors, among which are the compound, the species of plant, the diversity of genetic material, the plant part, and the environmental factors considered.

One of the problems attacked by the authors has been the breeding of tomatoes with higher content of vitamin C. Since tomatoes are available throughout the year, fresh or canned, and are a cheap popular food, it is anticipated that the development of varieties of higher vitamin C content would have a significant effect in overcoming the dietary deficiency of this vitamin which has been shown by a recent nutritional survey (1).

Work toward the development of varieties of tomatoes high in provitamin A (β-carotene) and ascorbic acid content was started at this station in 1942. The initial survey of commercial varieties and of noncommercial types, representing several species of *Lycopersicon*, for desirable parents of high vitamin content was reported earlier (7), and the progress toward the development of selections of high provitamin A content has been reported recently (6).

This paper reports the progress made to date toward breeding commercial varieties of tomatoes genetically constituted to contain a high content of ascorbic acid, and toward increasing the ascorbic acid content of possible parental material, and some observations on the inheritance of ascorbic acid.

Review of literature

HAMNER and MAYNARD (4) reviewed the literature prior to 1942 on the factors influencing the nutritive value of the tomato and concluded that the ascorbic acid content is dependent primarily on variety, on location where grown, and on seasonal conditions. REYNARD and KANAPAUX (10) reported, in 1942, on the ascorbic acid content of a large number of lines, including *Lycopersicon* species, foreign accessions, varieties, and progenies of segregating material. In the same year LINCOLN *et al.* (7) analyzed similar material. Both groups of workers found that the vitamin C content of selections within the species *L. peruvianum* (L.) Mill. and *L. pimpinellifolium* (Jusl.) Mill. had the highest ascorbic acid content of all the material surveyed. More recently, MCFARLANE *et al.* (8) and YEAGER and PURINTON (12) have reported on their progress in obtaining large-fruited selections having a high ascorbic acid content from segregating populations derived from crosses of *L. esculentum* Mill. with *L. peruvianum*. Other workers (2, 3, 11) have reported recently on the ascorbic acid content of varieties and hybrids and the influence of environmental factors on the ascorbic acid content of tomato fruits.

Material and methods

A modification of MORELL's procedure (9) for the determination of ascorbic acid in plant materials was used. A sample of at least 25 gm. of fruit was extracted with 100 ml. of either 1% metaphosphoric acid or 0.5% oxalic acid in a Waring Blendor for 2 minutes. From 1942 through 1945 metaphosphoric acid was used. In 1946 and 1947 oxalic acid was substituted for the metaphosphoric acid. The blended extract was filtered through no. 12 Whatman fluted filter paper, and a 25-ml. aliquot of the filtrate was titrated with a standard solution of sodium, 2, 6-dichlorobenzenoneindophenol. A moisture determination was made on each sample, and the dilution effect of the moisture in the sample was con-

sidered in calculating the ascorbic acid content.

With one exception, as noted later, all plants from which fruits were analyzed for ascorbic acid content were grown in the field. Open-pollinated seed was obtained from all plants tested for ascorbic acid content. Those selections to be used in crosses were taken to the greenhouse as cuttings in late September and grown to maturity. Selfed or crossed seed, as desired, was obtained in the greenhouse during the winter.

Experimentation and results

Correlations between ascorbic acid content and other characters

During the course of this work, attempts were made to correlate several characters with the ascorbic acid content of tomato fruits. Significant correlations were found, in certain instances, between ascorbic acid content and fruit weight, percentage of dry matter, and the lycopene and β-carotene content (table 1); but no character had a constant relationship with ascorbic acid content in all four of the genetically distinct groups studied.

The accession *L. pimpinellifolium* P.I. 126437 was found to be moderately high in ascorbic acid content. In 1943 a population of the parents and F_1 and F_2 populations of the cross, accession 126437 × Indiana Baltimore, were pot-grown in the greenhouse as a spring crop; similar populations were field-grown in the summer. The different environmental conditions under which the two series of populations were grown were reflected by a fruit size for the greenhouse populations of about 70% that of the field material (table 2). Greenhouse fruits, however, had an average of 44% more dry matter, 25% more ascorbic acid, and a greater variability, as indicated by the standard deviation of the mean ascorbic acid content. The differences were consistent for parental, F_1, and F_2 populations. In the F_1 plants small fruit size was almost completely dominant, and dry-matter percentage and ascorbic acid content were nearly those of the mean parental values for these characters. In the reasonably large F_2 populations significant negative correlations were observed between ascorbic acid content and fruit weight and fruit surface area; the latter value was calculated from measurements of the equatorial and polar diameters, assuming the fruit to be ellipsoidal. A significant positive correlation between ascorbic acid content and percentage of dry matter was also observed.

The *L. pimpinellifolium* accession 1796 was found to have the highest ascorbic acid content of all red- or yellow-fruited accessions tested. In 1943, F_1 populations of ten plants each of the cross, accession 1796 × Baltimore, and the reciprocal cross were grown in the field. Plants no. 7 and no. 2 had the highest ascorbic acid content in their respective populations. These plants produced the F_2 population and were used to produce the backcross populations which were grown in 1944, together with F_1 and parental populations. It should be emphasized that the F_2 and backcross populations were selected populations and do not give a true estimate of the variability of this cross. A summary of the data secured from this study is given in table 3. Again there was a definite dominance of small fruit size in the F_1. The ascorbic acid content was, however, only slightly above the mean of the parental values. The upper range of ascorbic acid content of the *L. pimpinellifolium* parent was not exceeded by any observation, but a single plant in a backcross population had less ascorbic acid than the lowest value of the commercial

parent. The negative correlation between ascorbic acid content and fruit weight was significant in all cases with the exception of the high ascorbic acid parent and in one backcross population.

In 1946 several populations segregating for ascorbic acid content, but with an average fruit size much greater than those studied in 1943 and 1944, were analyzed. These data are summarized in table 4. A highly significant negative correlation between ascorbic acid content and fruit weight was found in all the segregating populations.

TABLE 1

SUMMARY OF DATA SECURED IN SURVEY OF GENUS LYCOPERSICON, 1942

Description of material	Number of observations	Fruit weight (gm.)		Dry matter (%)		Ascorbic acid content (μgm/gm)		Correlation coefficient of ascorbic acid content with—			
		Range	Average	Range	Average	Range	Average	Fruit weight	Percentage dry matter	Lycopene	β-carotene
L. peruvianum	28	0.8–3.1	1.8	8.3–14.8	11.7	390–1050	788	0.003	0.233	−0.118	−0.183
L. pimpinellifolium	27	0.6–2.5	1.3	9.3–14.5	11.6	420–870	604	−0.208	0.733**	−0.345	0.443*
L. esculentum	86	3.0–266.0	59.3	4.0–16.2	7.1	100–666	395	−0.550**	0.873**	0.641**	0.134
F_2 [Baltimore × F_1 (Rutgers × *L. hirsutum*, P.I. 126445)]	88	2.0–189.0	44.2	4.1–10.7	6.2	100–490	256	−0.136	0.157	0.235*	0.259*

* Indicates significance at 5% level.
** Indicates significance at 1% level.

TABLE 2

SUMMARY OF DATA SECURED IN GREENHOUSE AND FIELD STUDIES OF PARENTAL, F_1, AND F_2 GENERATIONS OF CROSS L. PIMPINELLIFOLIUM P.I. 126437 × BALTIMORE, 1942

Description of material	No. of plants analyzed	Mean fruit weight (gm.)	Mean dry matter (%)	Ascorbic acid (μgm/gm)			Correlation coefficients of ascorbic acid content with—		
				Range	Average	σ	Fruit weight	Percentage dry matter	Fruit surface (sq. cm.)
L. pimpinellifolium P.I. 126437									
Greenhouse	11	0.7	14.6	640–890	725	71	−0.227	−0.533	−0.302
Field	20	1.1	9.5	409–590	532	18	0.209	0.078	0.141
L. esculentum var. Baltimore									
Greenhouse	8	82.4	6.8	230–440	351	66	0.063	0.578	0.203
Field	11	130.0	5.2	232–323	279	26	−0.456	0.030	−0.406
F_1 (P.I. 126437 × Baltimore)									
Greenhouse	9	6.2	11.8	470–620	530	46	−0.425	0.509	−0.507
Field	10	8.3	7.7	352–489	413	41	−0.427	0.839**	−0.365
F_2 (P.I. 126437 × Baltimore)									
Greenhouse	90	5.9	10.7	270–740	501	181	−0.470**	0.623**	−0.386**
Field	86	8.3	7.6	253–583	425	127	−0.415**	0.250*	−0.407**

* Indicates significance at 5% level.
** Indicates significance at 1% level.

TABLE 3

SUMMARY OF DATA SECURED IN FIELD STUDY OF PARENTAL, F_1, F_2, AND BACKCROSS GENERATIONS OF CROSS, ACCESSION 1796×BALTIMORE, 1944

Description of material	No. of plants analyzed	Fruit weight (gm.)		Ascorbic acid content (μgm/gm)			Correlation coefficients between fruit weight and ascorbic acid content
		Range	Average	Range	Mean	σ	
P_1 (1796-1)	19	0.44– 0.85	0.61	550–780	667	60	−0.225
P_2 (Baltimore)	20	93.0 –220.0	142.0	224–329	276	36	−0.480*
F_1 (1796-1×Baltimore)	19	3.0 – 59.0	4.3	418–635	518	68	−0.646**
F_1 (Baltimore×1796-1)	20	3.5 – 6.4	4.7	415–620	525	62	−0.582**
F_2 [plant 7 of F_1 (1796-1×Baltimore)]	80	0.7 – 13.5	5.4	301–716	491	86	−0.487**
F_2 [plant 2 of F_1 (Baltimore×1796-1)]	79	1.3 – 29.0	5.8	293–644	445	74	−0.414**
BC [plant 7 of F_1 (1796-1×Baltimore)×Baltimore]	40	5.4 – 49.4	24.8	208–507	359	73	−0.295
BC [plant 7 of F_1 (1796-1×Baltimore)×1796-1]	38	0.8 – 2.8	1.7	379–761	582	86	−0.353*
BC [plant 2 of F_1 (Baltimore×1796-1)×Baltimore]	40	8.0 – 62.2	20.1	247–510	377	66	−0.446**
BC [plant 2 of F_1 (Baltimore×1796-1)×1796-1]	40	0.9 – 6.2	1.8	369–774	550	46	−0.429**

* Indicates significance at 5% level.

** Indicates significance at 1% level.

TABLE 4

SUMMARY OF DATA SECURED IN FIELD STUDY OF TWO COMMERCIAL VARIETIES AND OF SEVEN POPULATIONS SEGREGATING FOR ASCORBIC ACID CONTENT, 1946

Description of material	No. of plants analyzed	Fruit weight (gm.)		Ascorbic acid content (μgm/gm)			Correlation coefficients between fruit weight and ascorbic acid content
		Range	Mean	Range	Mean	σ	
Baltimore	13	95–231	173	149– 378	261	55	−0.027
Rutgers	5	70–287	172	141– 318	243	70	−0.591
F_2 Sel. [Rutgers×F_1 (F_2 Sel. (1796-1×Balt.)×Balt.)]	129	24–138	67	115– 588	356	57	−0.324**
F_2 Sel. [Rutgers×F_1 (F_2 Sel. (1796-1×Balt.)×Balt.)]	116	4– 35	11	288–1040	569	105	−0.508**
F_2 Sel. [Rutgers×F_1 (F_2 Sel. (1796-1×Balt.)×Balt.)]	85	8– 70	39	302– 802	512	71	−0.600**
F_2 Sel. [Rutgers×F_1 (F_2 Sel. (1796-1×Balt.)×Balt.)]	93	3–130	51	228–1050	512	110	−0.420**
F_2 Sel. (1292-6-4[a]×Rutgers)	84	7– 50	25	286– 690	464	69	−0.315**
F_2 Sel. (1292-6-5×Rutgers)	97	7– 49	19	233– 630	456	76	−0.530**
F_2 Sel. (Rutgers×1292-6-5)	217	7– 72	14	275– 673	458	55	−0.485**

** Indicates significance at 1% level.

[a] 1292 is *L. pimpinellifolium* P.I. 119214.

SELECTION FOR INCREASED VITAMIN C CONTENT THROUGH SIX GENERATIONS OF A SEGREGATING POPULATION

Fruit of a selection of Purdue accession 1796, a *Lycopersicon pimpinellifolium* stock received from Dr. L. J. ALEXANDER and carrying the Ohio Station number C2-9-2, had an ascorbic acid content of 780 μgm/gm, the highest value found for the red- and yellow-fruited accessions tested at this station. The ascorbic acid content of Indiana Baltimore fruits analyzed in 1942 averaged 265 μgm/gm. Although accession 1796 has an ascorbic acid content about three times that of Indiana Baltimore, the large amount of ascorbic acid found in the commercial variety indicates that the latter must contain some genes of importance in ascorbic acid synthesis. If the higher ascorbic acid content of the *L. pimpinellifolium* accession resulted from the same factors as those present in Indiana Baltimore, plus an additional gene complement influencing ascorbic acid content, it would not be expected that progeny of such a cross would exceed the range of that of the high vitamin parent. To determine whether progeny of higher ascorbic acid content than that of the high vitamin parent could be obtained, a selection experiment was conducted. This experiment would have been of greater genetic interest if selection toward a low

TABLE 5

ASCORBIC ACID CONTENT AND FRUIT WEIGHT OF PARENTS AND ADVANCED GENERATIONS OF CROSS 43403, BALTIMORE×ACCESSION 1796

YEAR	PEDIGREE (SELECTION OR VARIETY)	GENERATION	NO. OF OBSERVATIONS	FRUIT WEIGHT (GM.)	ASCORBIC ACID CONTENT (μGM/GM)	
					Mean or range	σ
1942	1796	P	1	1	865	
	Baltimore	P	7	170	220	63
1943	1796-1	P	22	1	684	81
	Baltimore	P	32	143	272	46
	43403F7	F_1	1	2	540	
	Range of 10 F_1 plants		10	1–3	390–560	
	Mean of 10 F_1 plants		10	2.4	476	65
1944	1796-1-2	P	19	1	667	60
	Baltimore	P	20	142	276	36
	43403F7-3	F_2	1	5	707	
	Range of 80 F_2 plants		80	1–14	301–716	
	Mean of 80 F_2 plants		80	5.3	497	86
1946	1796-1-2-3	P	3	1	902	
	Baltimore	P	13	173	261	55
	43403F7-3-18-10-5	F_5	1	15	920	
	Range of 6 F_5 plants		6	13–20	557–930	
	Mean of 6 F_5 plants		6	16.7	710	85
1947	1796-1-2-3-3-X	P	1	1	890	
	Baltimore	P	10	160	380	64
	43403F7-3-18-10-5-X	F_6				
	Range of 27 F_6 plants		27	7–51	625–1067	
	Mean of 27 F_6 plants		27	12.3	915	107

ascorbic acid content had also been made. Except for the F_3 population grown in the greenhouse, all populations were grown in the field, and all seed saved was from open-pollinated flowers. Each year several selections from the pedigree that had proved to be the highest in ascorbic acid content of all progenies tested in the previous year were grown. A variable number of analyses for ascorbic acid content was made on fruit of the individual plants of each sibling population. After analyses were completed, all populations except the one with the highest average ascorbic acid content were discarded, and from this population several sib plants of the highest ascorbic acid content were selected for planting the following year. Selection for high ascorbic acid content has been continued into the sixth generation. The results of selection are presented in table 5. Selections containing a higher ascorbic acid content than the high ascorbic acid parent have been found, indicating that each parent probably contributes some genes not found in the other parent.

The average percentage of outcrossing in the tomato is less than 5%. Consequently, this work can be considered as done within selfed lines, and, therefore, genetic variability would be reduced by one-half in each generation. Yet, in this experiment the variability in the F_2 was about 17% of the mean and was still 12% of the mean in the F_6 generation. This variability is about the same as that of the parental varieties. It would appear that many genes are concerned in the production of a high ascorbic acid content in tomatoes and that environmental factors greatly affect the ascorbic acid content of a selection.

Selections obtained as a result of this experiment are proving particularly valuable in the varietal breeding program; the best F_1 plants of the cross of certain F_5 selections and Rutgers had a fruit weight of 156, 104, and 106 gm. with an ascorbic acid content of 533, 592, and 608 μgm/gm, respectively.

Development of large-fruited high ascorbic acid tomatoes

Two varieties, Rutgers and Indiana Baltimore, are grown to the exclusion of almost all other varieties of canning tomatoes in Indiana. Consequently, these varieties have been used as recurrent parents in the breeding program. Otherwise no standard procedure has been followed. About twenty different high vitamin accessions, all of them small-fruited types of the species *L. esculentum* or *L. pimpinellifolium*, have been used as possible high ascorbic acid parents in crosses with Rutgers or Indiana Baltimore. In the early segregating generations from these crosses the high ascorbic acid selections were small-fruited. When these selections were crossed, Indiana Baltimore or Rutgers was used as the recurrent parent to increase fruit size and to make possible the selection of a vine type similar to the commercial parent. As the fruit size of the selections increased to 80 gm. or more, crosses between high ascorbic acid selections were made. In 1947 fruits of about sixteen hundred plants were analyzed for their ascorbic acid content. Accession 1796 was the genetic source of high ascorbic acid content of 80% of the best one hundred and fifty selections, whereas about 8% had accession 1292 (P.I. 119214) as the high vitamin parent. Approximately 6% were derived from crosses between selections originally derived from accessions 1796 or 1292, and about 3% had a *L. hirsutum* accession as the possible source of high ascorbic acid. Fruit size and ascorbic acid content of certain of these selections

and of the commercial varieties are given in table 6. Fruit size still does not equal that of the varieties, but the ascorbic acid content is almost twice that of the commercial parents.

Ascorbic acid content of L. peruvianum selections

Fruits of certain accessions of the species *L. peruvianum* had the highest concorbic acid selections from the breeding program have not been successful. Sterile triploid plants were obtained, however, from the cross 4 N *L. pimpinellifolium*—obtained from Dr. E. W. Lindstrom, Ames, Iowa—with three different selections of *L. peruvianum*. It may reasonably be expected that, when the desired crosses are obtained, the ascorbic acid content of red-fruited segregates will be

TABLE 6

Vitamin C content and fruit size of some selections and two varieties grown in 1947

Pedigree or variety	Generation	Fruit weight (gm.)	Ascorbic acid content (μgm/gm)
Baltimore (Indiana Canner's Strain)	P	160	380
Rutgers	P	200	360
(1796-1×Balt.) F_2 Sel.×Rutgers	F_1	159	558
(1796-1×Balt.) F_2 Sel.×Rutgers	F_1	149	542
(1796-1×Balt.) F_2 Sel.×Rutgers	F_1	121	567
(1796-1×Balt.) F_2 Sel.×Rutgers	F_1	123	542
(1796-1×Balt.) F_5 Sel.×Rutgers	F_1	156	533
(1796-1×Balt.) F_5 Sel.×Rutgers	F_1	104	592
(Rutgers×1292-6-5) F_2 Sel.×Balt.	F_1	147	500
(Rutgers×1292-6-5-) F_2 Sel.×(1796-1×Balt.) F_2 Sel.	F_1	105	725
(Pan American×F_1 (Pan American×*L. hirsutum* P.I. 127826) F_3 Sel.×(Balt.×F_1 (Rutgers×*L. hirsutum* P.I. 126445) F_3 Sel.	F_2	94	617
Rutgers×(1796-1×Balt.) F_2 Sel.	F_3	146	525
Rutgers×(1796-1×Balt.) F_2 Sel.	F_3	107	750
Rutgers×(1796-1×Balt.) F_2 Sel.	F_3	106	742
Rutgers×(1796-1×Balt.) F_2 Sel.	F_3	54	858
Rutgers×(1796-1×Balt.) F_2 Sel.	F_4	30	1033
Rutgers×(1796-1×Balt.) F_2 Sel.	F_4	35	958
Average of all selections		109	665

tent of ascorbic acid of any *Lycopersicon* type included in the initial 1942 survey. Selections within this species for high ascorbic acid types of possible value in a breeding program have continued. In 1946 about two hundred analyses were made of several selections representing this species (table 7). The highest content of ascorbic acid found in these selections is about one-half greater than that of any red-fruited selection thus far obtained. Attempts to cross selections from the species *L. peruvianum* with high asgreatly increased. It is possible that another valuable characteristic might be obtained from such crosses, for we have noted that the ascorbic acid content of *L. peruvianum* types does not decrease in the latter part of the growing season as it does in red-fruited types. After a period of cool, cloudy weather, such as may occur in this area in late September and October, the ascorbic acid content of the red-fruited types becomes extremely variable. The ascorbic acid content of *L. peruvianum* selections after a similar pe-

riod of cool, cloudy weather remains high.

Modification of method of analysis for ascorbic acid

In routine breeding work only the best 1–5% of the material analyzed is useful as parental material. If a method whereby 90% of the individual plants to be analyzed—those with the lowest ascorbic acid content—could be discarded by a gross but rapid method of analysis, the remaining material of relatively high ascorbic acid content could be more thoroughly studied in a given time. Such a method has been developed, but it has not been compared with the standard method on a scale sufficiently extensive to permit a complete evaluation.

By this method fruits were quick-frozen at −20° C. After the fruit was frozen solid, fruits were placed in beakers in a vacuum oven until no longer frozen (2 hours at 50° C.). If thawed in the atmosphere of a laboratory, moisture condenses on the fruit, thereby diluting the juice that exudes from the fruit on thawing. The free juice was filtered, and 5 ml. of the undiluted juice were titrated with standardized dye. The samples analyzed by this modified method have averaged about 8% higher in ascorbic acid content than the duplicate samples run by the routine method. A few samples were held frozen for 2 weeks and did not vary significantly from samples analyzed soon after becoming completely frozen.

Discussion

Tomato selections have been obtained with fruit size of about 5 ounces (140 gm.) which contain more than 500 μgm/gm of ascorbic acid. The ascorbic acid content of such fruits about equals that of citrus fruits. The adult minimum daily requirement of this vitamin would be furnished by 55 gm. of fresh fruit, whereas 135 gm. would furnish the recommended daily allowance for an active man (5).

Little new information has been presented on the inheritance of ascorbic acid productivity in tomatoes. Genetic differences among lines exist. The ascorbic acid content of the F_1 hybrid between high and low ascorbic acid lines approximates the mean content of the parents.

TABLE 7

Range of ascorbic acid content and fruit size of L. peruvianum accessions, 1946

P.I. accession	Fruit size (gm.)	Range of ascorbic acid content (μgm/gm)
889	3.0	944–1489
901	1.5	636–1116
903	2.2	912–1596
904	2.8	882–1596
910	1.8	585–1061
917	1.5	758–1458
1507	1.7	703–1305
1508	1.3	885–1290
1511	2.2	887–1272
1512	1.5	793–1447
1514	1.6	713–1054
1515	1.7	723–1280
1516	1.4	710–1234
1521	0.8	1188–1317
1967	1.1	1037–1532

There is no evidence of transgressive segregation in F_2 populations of less than one hundred plants, and, in these data, the F_2 distribution was normal. Selection of fruit for high ascorbic acid content through six generations has produced selections with 20% more ascorbic acid than the original parent and with a fruit size about twenty times greater than the high ascorbic acid parent. A statistically significant negative correlation between ascorbic acid content and fruit size has been observed in almost all populations derived from crosses between *L. esculentum* and *L. pimpinellifolium*. The average correlation statistic was about

$r = -0.45$. This is a relatively small correlation value, regardless of statistical significance, and would indicate that many exceptions to the association of these characters occur. It also indicates that other factors not taken into account by these data are important in determining the ascorbic acid content of a fruit. The increase in the mean value of ascorbic acid in advanced populations selected for high ascorbic acid content shows that the genetic constitution of the population is the most important physiological factor in determining the ascorbic acid content of a particular plant or population, although other variables may influence that character. All information on the inheritance of ascorbic acid content indicates a multiple factor type of inheritance. The fact that the F_6 population still has a standard deviation equal to about 12% of the mean ascorbic acid content of that population, even though the mean ascorbic acid content of the F_6 is higher than the high parent, indicates either that many genes produce an effect on ascorbic acid content or that factors other than genetic are responsible for this variability. Probably both genetic and environmental variance is being measured.

Summary

1. A negative correlation between ascorbic acid content and fruit size usually was observed in these studies. Observations were made on the species *Lycopersicon peruvianum*, *L. pimpinellifolium*, *L. esculentum*, and the segregating populations of crosses between *L. esculentum* and *L. pimpinellifolium* and *L. esculentum* by *L. hirsutum*. In fourteen segregating populations derived from crosses between very small-fruited selections of high ascorbic acid content with large-fruited commercial varieties of low ascorbic acid content, a significant negative correlation of approximately −0.45 between fruit size and ascorbic acid content was observed. No constant relationship between ascorbic acid content and percentage of dry matter, lycopene, or β-carotene content was observed.

2. Selections with a higher ascorbic acid content than the high ascorbic acid parent have been obtained in advanced generations of segregating populations. It appears that many genes are involved in the inheritance of a high ascorbic acid content.

3. Selections with an average fruit size of 150–160 gm. and an ascorbic acid content of 530–560 μgm/gm have been obtained. Such selections have fruit size slightly smaller than commercial canning varieties but contain about twice as much ascorbic acid.

4. *L. peruvianum* selections with an ascorbic acid content as high as 1600 μgm/gm have been obtained. This content of ascorbic acid is one and one-half times greater than that found in any red-fruited selection and from four to five times greater than that of commercial varieties.

5. A rapid modified method of analysis for ascorbic acid has been described.

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GERMINATION AND GROWTH INHIBITORS FROM RED KIDNEY BEAN SEED[1]

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY **612**

S. M. SIEGEL

Experimentation

In the course of investigations on the light sensitivity of various seeds, it was observed that the Red Kidney bean (*Phaseolus vulgaris*) showed germination percentages as high as 95 in Petri dishes kept in darkness but only 5–15% in dishes in bright sunlight (maximum 4000–6000 foot-candles). The effect was more pronounced at 30°–32° C. than at 20°–25° C., suggesting the presence of a chemical inhibiting system. Experiments were therefore begun to test for the presence of chemical inhibitors of germination and growth in various parts of the Red Kidney bean seed.

At first, the effect on germination only was tested. Grains of spring wheat were allowed to germinate in Petri dishes on filter paper saturated with either distilled water or an extract made by agitating coarsely broken whole-bean seeds (50–100 gm/liter) with distilled water at about 15° C. for 1–3 hours. Eight samples of twenty-five grains each were germinated in bright sunlight with a maximum intensity of about 4000 foot-candles, four with water only, four with the extract; corresponding sets were germinated in darkness. All groups were incubated at 24°–27° C. After 72 hours, of those lots germinated in light, controls with water averaged 80% germination, those with the extract about 50%; in darkness controls averaged 92%, those with extract 82%. When an extract made by soaking 0.5 gm. of bean seed coat in 25 ml. of distilled water for 2 hours was used, germination of the wheat was found to be about 90% for controls and 70% for groups with the extract after 60–70 hours in either light or darkness at about 26° C.

When whole Red Kidney beans, soaked in water for 1 hour and then ex-

[1] This work was supported in part by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.

posed to sunlight of 2000–3000 foot-candles intensity for 3 hours, were germinated in darkness, the percentages at 24, 48, and 72 hours were 6, 54, and 80; 4 controls soaked but not irradiated gave values of 50, 78, and 90% for these test intervals. If, however, whole-bean extract, such as was employed with the wheat, either unirradiated or irradiated with 2000–3000 foot-candles of sunlight for 3 hours, was applied to unirradiated, unsoaked beans in darkness, no effect on germination was observed.

TABLE 1

EFFECT OF VARIOUS BEAN-SEED EXTRACTS ON GERMINATION AND GROWTH OF FLAX

Source of extract	Treatment of extract		Control with water
	Irradiated	Unirradiated	
	Percentage germination*		
Whole bean	20	39	70
Seed coat	70	75	70
Cotyledons	72	68	84
	Length of flax roots (mm.) after 80 hours†		
Whole bean	5.8	14.7	21.9
Seed coat	12.7	13.5	21.3
Cotyledons	17.8	7.5	23.2

* Each value based on 80–100 seeds.

† Each value based on 40–50 seedlings.

The percentage germination of flax after 70–80 hours in contact with various bean extracts is shown in table 1. All extracts were prepared in the dark by soaking broken-up whole beans or their parts in distilled water (10–20 gm/liter) with agitation for about 3 hours. Extracts either were then exposed to 4000–5000 foot-candles of sunlight for 3 hours or were unirradiated (both groups maintained at about 26° C.). The extracts were then applied to the seeds, 2 ml. per 25–30 seeds, and germination was allowed to proceed at an incubation temperature of 24°–26° C. It is clear that the whole-bean preparation was far more active than the others and that its inhibitory effect was increased somewhat by irradiation. The cotyledon extract may or may not have had a slight effect but was not activated by the light employed.

These same extracts caused some inhibition of root growth in flax grown at 25° C., but the activity of the seed-coat extract in affecting root growth was unaltered by light, and the cotyledon extract was partially inactivated by irradiation (table 1). Only in the case of the whole-bean extracts was there found a parallel effect on germination and root growth. When the various extracts were boiled before application to the flax seeds, the activity of the seed-coat extract was diminished, that of the others was unaltered. Only 10–15 minutes of either direct boiling of the extracts or treatment in a water bath at 100° C. was needed to inactivate almost completely the seed-coat extract. Root growth in test plants to which the boiled extract was applied did not differ significantly from that in controls with water.

The growth of the primary root of wheat was affected considerably, and that of the coleoptile slightly, by bean seed-coat extract (table 2). Plants were grown for 3 days in Petri dishes, with 2 ml. of extract per dish, at 23° C. All extracts inhibited root growth, but the boiled irradiated extract was only half as effective as the unboiled irradiated; and the boiled unirradiated extract was found to be even less active than the boiled irradiated one (data not given in present paper). Irradiation had only a slight activating effect. In action on the coleoptile

the irradiated extract was most inhibitory, the unirradiated one slightly stimulatory. The only difference of significance, however, was that between irradiated and unirradiated preparations.

Several other tests and test plants were employed, including alfalfa seedlings and young sunflower plants. Although the responses of these test plants again indicated the presence of inhibitors in bean seeds, the tests are not sufficiently standardized, at present, to merit the inclusion of results obtained in this paper.

The writer was unable to extract active substances from bean seeds with solvents other than water—such as ether, alcohols, acetone, or various hydrocarbons.

Neither ultraviolet nor infrared radiation proved to be effective in activating those preparations activated by sunlight. In sunlight or artificial white light the whole-bean extract was observed to change in color from a yellow- or orange-brown to a dark brown; the significance of this color change with respect to the extract's biological activity is not known at present.

Discussion

In his review of germination inhibitors, EVANARI (3) did not mention the seed of the Red Kidney bean, although several references were made to light-activated substances in other cases. CROCKER (2), in reviewing the literature on light sensitivity, cited several cases of specific chemical inhibitors being detected; some of these were claimed to be photodynamic in nature. BARTON and SOLT (1) recently reported inhibition of growth by extracts of seeds of pole bean varieties (*Phaseolus sp.*). Using the growth of the primary root of wheat in water culture as the test, they found the greatest inhibitory activity in aqueous extracts of the beans and a greater activity in the seed coat than in the cotyledons or embryonic axis of the seed. No mention was made of the activation by light of an inhibiting principle. The slight effect on activity of boiling is mentioned in their paper. In the present work the whole-bean extract was found to be the most active, and boiling for 10–15 minutes decreased the activity considerably in some cases. The experimental conditions differed, however, and these results cannot be properly compared with those of BARTON and SOLT.

TABLE 2

LENGTH (MM.) OF PRIMARY ROOT AND COLEOPTILE OF WHEAT GROWN IN BEAN SEED-COAT EXTRACT FOR 3 DAYS*

ORGAN	TREATMENT OF EXTRACT			
	Irradiated	Boiled irradiated	Unirradiated	Control with water
Root	21	42	29	51
Coleoptile	32	39	43	38

* Each value based on 20–25 seedlings.

The differences evident in the germination of flax in contact with various extracts would seem to suggest that the whole bean contains a germination inhibitor not present in isolated seed coats or cotyledons. Growth data, on the other hand, indicate inhibiting substances in the seed coat, cotyledon, and whole-bean extracts. Inasmuch as each extract differed in its response to irradiation, it is possible that at least three inhibitors occur in the bean seed. No explanation can be given for the opposite effect of light on the whole-bean and cotyledon extracts save that some substance or system in the whole bean, in being activated by light, may serve to protect the cotyledon inhibitor from photoinactivation.

The simultaneous inhibition of root growth and slight stimulation of coleoptile elongation in wheat by unirradiated seed-coat extract is suggestive of an auxin-like action of some substance. The decrease in inhibitory activity by irradiation of the cotyledon extract (table 1) is not inconsistent with the assumption that the inhibitor in the extract is an auxin. On the other hand, the response of the cotyledon extract to irradiation is somewhat similar to that of coumarin-treated lettuce seeds (4), the germination of which was inhibited by the coumarin in the dark but not in the light.

Summary

Extracts of Red Kidney bean seeds, untreated or treated by boiling or by irradiation with sunlight, inhibited germination and growth in flax, wheat, and other test plants. Irradiation activated, and boiling inactivated, certain extracts. Only aqueous extracts were effective; only visible light increased inhibitory activity. The varying properties of extracts from different parts of the bean seed suggest that more than one inhibiting principle is present.

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AGE OF AMERICAN COAL-BALL PLANTS[1]

It seems desirable to point out that the geologic age of specimens of *Sphenophyllum* and *Psaronius* recently described by Professor FREDDA REED (6) probably is not so restricted as she suggested. She quotes NOÉ as saying of the Indiana, Illinois, and Texas coal balls: "They are all from coal fields which are part of the Allegheny formation of the Pennsylvanian system." Evidently some misapprehension has occurred, as it is well known that some of NOÉ's Illinois collections and the coal balls available to him from Texas are from beds younger than the Allegheny formation.

The writer (7) has previously reviewed the stratigraphic occurrence of Illinois and Indiana coal-ball horizons. Since then some plants have been reported by REED from Texas coal balls (5), and recently the writer has further checked on the source of this material and made new collections. The only Texas coal balls known come from mines on the outskirts of Newcastle in Young County. NOÉ (3) recorded that specimens from Newcastle had been sent him by W. E. WRATHER. It has long been recognized that the Newcastle, Texas, coal is of uppermost Pennsylvanian (Virgil) age (4, cf. p. 160; 1, cf. p. 65), and there can be no doubt that plant fossils from these coal balls are considerably younger than others known from coal balls elsewhere in this country.

REED does not give particular source localities for any of the specimens described in her recent paper. Hence one must conclude that any of these materials may be from beds as young as the Newcastle coal of Texas or as old as the Silver Island coal, the oldest from which NOÉ is known to have collected coal balls in Illinois or Indiana. According to the Report of the National Research Council on correlations of Pennsylvanian formations in North America (2) this age range, in terms of the Appalachian succession, extends from the time of deposition of beds somewhat older than the Allegheny, equivalent to the younger part of the Kanawha formation, to beds younger than the Allegheny and succeeding Monongahela formations, and includes

[1] Published by permission of the Director, United States Geological Survey.

all but the youngest part of the Conemaugh. In any careful evaluation of the evolutionary characters of plants such a breadth of age span can make a considerable difference.

All published reports about fossil plants have a bearing on the paleontologic and historical aspects of geology, and no report dealing with fossil plants should be considered as applying to botany alone. This note, in addition to constituting a correction of the possible ages of the *Sphenophyllum* and *Psaronius* material reported by REED, should also be interpreted more generally as a plea for accurate reporting of sources for fossil plants. Such data have an important relation to the historical interpretation of plant life, regardless of their classification as a contribution to botanical or to geological knowledge.

It should be appreciated that age assignment based on paleontologic materials requires extensive knowledge and keen judgment in evaluating relative evidence. A botanist reporting on fossil plants may or may not feel inclined to undertake the problem of their precise age assignment and, in fact, should not be tempted to indulge in age speculation gratuitously. However, objective facts regarding the source of material, the local name for the source bed or reference by distance above or below some distinguishable locally named bed or horizon, and, above all, a description of the geographic locality are essential to any scientific reporting of fossils. Such details are much more important for reference in studies of fossil plants than they are even for systematic study of the modern flora. Plants of the modern flora can represent only an insignificant and essentially momentary time span in comparison with the span of ages transgressed by the sedimentary rocks of different localities. For example, the period of time represented by beds of Allegheny age alone may be on the order of 5 to 10 million years; the span of time from deposition of the upper part of the Kanawha formation to time of closing deposition of the Monongahela formation must be three or four times as long. No accurate absolute chronology for subordinate stratigraphic units is possible, of course, for beds of this antiquity, but the relative ages of subdivisions of the Pennsylvanian are established beyond any doubt. More accurate relative knowledge of the plant lineages and of the time succession must be pieced together from collections and records of precise location.—JAMES M. SCHOPF, *U.S. Geological Survey, Coal Geology Laboratory, Orton Hall, Ohio State University, Columbus 10, Ohio.*

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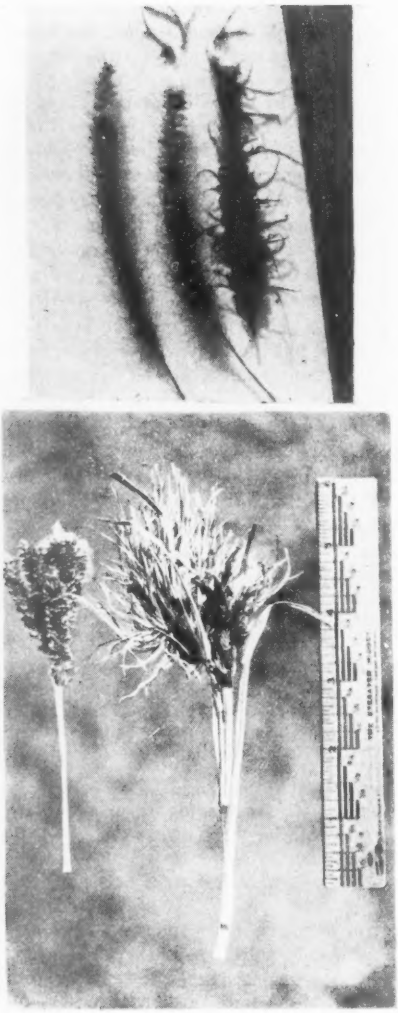
VIVIPARY IN SOME CHINESE PLANTS[1]



FIGS. 1, 2.—Vivipary in *Setaria viridis* (fig. 1, *above*) and *Eleusine coracana* (fig. 2, *below*). Normal heads on left, viviparous heads on right. (Photographs by Mr. FANG CHI.)

Vivipary, the phenomenon of seeds germinating while still attached to the parent plant (2), is known in many families of seed plants. The classic example is the mangrove (*Rhizophora*). Members of the Agavaceae, such as *Agave americana* and *Phormium tenax*, also show this ability. In Java the author has observed cases of vivipary in the mango (*Mangifera indica*), and vivipary in this species has been recorded from India (5). Vivipary in the vegetable pear (*Sechium edule*) is a common occurrence in Java. In fact, one method of propagating the vegetable pear is to plant a vivipar. In southeastern China it is not uncommon to find longan (*Euphoria longana*), litchi (*Litchi chinensis*), and *Citrus* seeds showing signs of sprouting in ripe fruits. But actual cases of vivipary in these plants have not been observed.

Among the grasses, such as *Poa bulbosa*, and in many members of the rush family, vivipary occurs mostly in high latitudes and at high altitudes, mainly above those at which these plants normally flower (1). Artificial vivipary has been induced by controlled short photoperiod in *Deschampsia flexuosa* at the Alpine Botanical Garden, Kola Peninsula (4). Induced vivipary was also reported in varieties of common barley (3).

On August 10, 1948, after a considerable period of drought, five plants representing two species of wild grasses, *Setaria viridis* (fig. 1) and *Eleusine indica*, were found bearing plantlets on the seed heads. These plants were growing near the edge of a tennis court on the level grounds of Fukien Christian University, Foochow, China. This discovery led to a survey of all cultivated grass species on the experimental farm of the College of Agriculture, and on September 1, 1948, a small bed (4′ × 100′) of *Eleusine coracana* (fig. 2) was found to have half of its population showing vivipary, mostly on the side shoots of the culms. Data on viviparous plants of the three species are given in table 1.

Two viviparous plants of *S. viridis* were potted on August 10 and kept for further observation of the behavior of the vivipars on the heads of the mother plants. Some of the vivipars grew to a height of 6 cm., although the majority

[1] Contribution jointly from Fukien Christian University, Foochow, China, and The British Imperial Chemical Industries Co. (Ltd.), England.

were 3–4 cm. tall. On September 2 several had bloomed and were forming seeds.

E. coracana is an economic plant in Foochow as well as in other parts of Fukien. It is generally used for food. Seeds are sown about the middle of April. Two harvests are possible. The first crop is usually harvested at the end of July, after which young culms and leaves spring up from the stubble and bear mature heads toward the end of September or the middle of October. No case of vivipary was observed in the first crop, but a number of side shoots became viviparous during the second crop. A number of viviparous plantlets were separated from the mother plants and, when planted in soil in a pot, grew readily.

Although the factors inducing vivipary in these plants in 1948 in Foochow are not known, the pronounced drought conditions of summer and autumn might have been a controlling factor.

ALLAN and CRANWELL (1), working with grasses in New Zealand, thought that altitudes affect vivipary. On September 15, 1948, accompanied by several of my students, I made observations on *S. viridis* and *E. indica* from close to sea level to an elevation of 919 meters (Kushan Peak) but failed to find any viviparous plants at higher altitudes.—LAI-YUNG LI, *College of Agriculture, Fukien Christian University, Foochow, China.*

TABLE 1

NATURAL VIVIPARY IN SETARIA VIRIDIS, ELEUSINE CORACANA, AND E. INDICA

Species	Plant no.	Total no. heads	No. viviparous heads	Av. no. plantlets on viviparous heads
S. *viridis*	1	36	3	103
	2	40	3	96
	3	1	1	80
	4	1	1	82
E. *indica*	1	12	1	12
E. *coracana*	1	12	1	32
	2	15	4	72
	3	18	3	25
	4	15	4	68
	5	9	1	28

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CURRENT LITERATURE

Practical Plant Anatomy. By ADRIANCE S. FOSTER. 2d ed. Toronto, New York, London: D. Van Nostrand, Inc., 1949. Pp. xi+228. $3.00.

This new edition of FOSTER's *Anatomy* follows the same general pattern of organization and is written to serve the same purposes as the first edition. It has, however, been expanded in many areas and brought up to date throughout. The number of references given with each exercise has been increased, an important feature in a text which attempts to give only the highlights of anatomical fact and theory.

The instructor of a course in plant anatomy is faced with a problem in the selection of a text. FOSTER's book is clearly written, compact, and, in the opinion of this reviewer, maintains an admirable balance between historical, modern, and possible future developments in the field. The dynamic aspects of anatomy as shown in ontogenetic and experimental morphological studies, and in the correlation of structure to function, are brought to the fore, thus making the book more interesting reading and more challenging to the student. In situations, however, where a single text is desired, and facilities for access to supplementary readings are inadequate, the briefness of the text and the absence of illustrations in *Practical Plant Anatomy* are drawbacks. Even if using another book as a class text, an instructor would find a personal copy of this book very useful for its suggestions as to suitable material for illustrating certain points and as to techniques helpful for preparation of many of the materials.

There are certain areas of the subject matter (not regions of major importance) which appear to this reviewer to be misplaced or slighted, while the treatment of others (of much greater importance) is exceptionally good. In the former category would be included the treatment of the endodermis, secretory cells or tissues, root-stem transition, and the noninclusion of paradermal leaf sections for helping the student understand the three-dimensional aspects of the structure of the mature leaf, particularly in reference to the functions of the leaf. Discussion of the endodermis in any detail is found only in connection with root anatomy and not with the other cell types, though it is mentioned as occurring in many stems and leaves. Although its appearance is not universal, its ontogenetic origin undetermined in many instances, and its function in the plant not clearly understood, the endodermis is no more deficient in these respects than some other cell types discussed independently. Although the exercise on laticiferous tubes is well done, other cells or tissues often classed with them as "secretory tissues"—such as resin canals, glandular hairs, oil glands, nectariferous tissue, or hydathodes—are mentioned only in passing or omitted completely. The work on the "transition" region of *Daucus* is highly illuminating, but it seems inadequate by itself to lead to the discarding of the earlier works. Admittedly the terminology "twisting" and "inversion" is poor, but if the vascular strands are studied with attention to a possible gradual and continuous reorientation of the direction of maturation of the tracheary elements, for example, it may be that much of the detailed work on transition regions can be s· lv·ged.

In the latter category mentioned above (i.e., areas which are very well covered) would be included the discussion of tracheids and vessel elements, sieve cells and sieve elements, and those portions which discuss the shoot apex and the ontogeny of the stem and leaf. There have been so many new papers in these areas in recent years that a clear, concise discussion which includes the important information from these papers, such as is found in this book, may not only keep the student from becoming thoroughly befuddled but will also help the instructor in planning how best to present the matter to the student.—B. F. PALSER.

Mosses of Guatemala. By EDWIN B. BARTRAM. Fieldiana: Botany 25:i–v, 1–442. 1949. 190 figs. Chicago Natural History Museum Pub. 621. $4.00 plus postage.

Within a period of less than 11 years the mosses upon which this monograph is based were not only collected by Drs. PAUL C. STANDLEY and JULIAN A. STEYERMARK in the more tropical departments and by Dr. A. J. SHARP in the highlands of Guatemala but they were also identified and verified and the pertinent data concerning them was assembled in final form by Mr. BARTRAM. Two hundred and five genera are represented by 519 species. At the higher elevations many of the mosses common in our northeastern states grow luxuriantly. Only 11% of the species are endemic or described as new.

Excellent in every detail of presentation and typography, this volume now ranks first among the bryological floras of tropical America. It is a real contribution to the taxonomy and phytogeography of the Western Hemisphere.—PAUL D. VOTH.